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الجامعة الأردنيـة
كلية الدراسات العليا

التاريـــخ: ١٨/ ٨/ ٢٠١١م

نموذج رقم (١٨)
اقرار والتزام بقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الماجستير

أنا الطالب: اشرف فتحي جاد الله الربيع الرقم الجامعي: 8080963
التخصص: علوم الصيدلة الكلية: الصيدلة

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد رسائل الماجستير والدكتوراة عندما قمت شخصيا" باعداد رسالتي / اطروحتي بعنوان:
Evaluation of Salvia triloba methanolic extract as potential anti-Hypertriglyceridemic agent
تقييم فعالية نبتة الميرمية لاختبار فعاليتها كعامل خافض للدهنيات الثلاثية

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توقيع الطالب: اشرف الربيع التاريخ: ١٨/ ٨ / ٢٠١١م

EVALUATION OF *SALVIA TRILOBA* METHANOLIC EXTRACT AS POTENTIAL ANTI-HYPERTRIGLYCERIDEMIC AGENT

By

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This Thesis was submitted in Fulfillment of the Requirements for Master's Degree in Pharmaceutical Science

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COMMITTEE DECISION

This Thesis (Evaluation of *Salvia triloba* methanolic extract as potential anti-Hypertriglyceridemic agent) was Successfully Defended and Approved on 7/8/2011.

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Dedication

I WOULD LIKE TO DEDICATE THIS WORK TO

MY BELOVED WIFE AREEJ FOR HER SUPPORT, PATIENCE, LOVE AND HELP

TO MY SWEET KIDS ZAID AND QAIS

TO MY BELOVED PARENTS

TO MY BROTHERS FIRAS, ANAS, MOTASEM, MUHANNAD, AND MO'AYYAD

TO MY SISTERS

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List of Abbreviations

Abbreviation	Definition
ADA	American Diabetes Association
ADP	Adenosine DiPhosphate
AHI	Apnea-Hypopnea Index
ATP	Adenosine TriPhosphate
BMI	Body Mass Index
CVD	Cardio Vascular Disease
DHAP	DiHydroxyAcetone Phosphate
DMSO	DiMethyl SulphOxide
EC	Enzyme Commission number
FAO	Food and Agriculture Organization
FFA	Free Fatty Acids
GC	Gas Chromatography
GLUT-4	Glucose Transporters-4
GPO	GlyceroPhosphate Oxidase
G-3-P	Glycerol-3-Phosphate
HDL	High density Lipoprotein
IFG	Impaired Fasting Glucose
IGF-1	Insulin-like Growth Factor 1
IGT	Impaired Glucose Tolerance
IL-6	Interleukin- 6

L.	Labiatae, Lamiaceae
LDL	Low Density Lipoprotein
LPa	LipoProtein a
LPL	LipoProtein Lipase
MCP-1	Monocyte Chemotactic Protein-1
MI	Myocardial Infarction
mRNA	messenger RiboNucleic Acid
NHANES III	National Health and Nutrition Examination Survey III
OA	Oleanolic Acid
OHS	Obesity Hypoventilation Syndrome
OSA	Obstructive Sleep Apnea
PAI-1	Plasminogen Activator Inhibitor-1
PAR	Population Attributable Risk
PL	Pancreatic lipase
POD	Peroxidase
PNPB	Para-NitroPhenyl Butyrate
PPL	PorcinePancreatic lipase
PPAR- γ	Peroxisome Proliferator Activated Receptor-γ
RAAS	Renin-Angiotensin-Aldosterone system
ROS	Reactive Oxygen Species
S.	Salvia
TG	TriGlyceride
TNF-α	Tumor Necrosis Factor-α
UA	Ursolic Acid
VLDL	Very Low Density Lipoprotein

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Abstract

Background:Sage (*Salvia triloba*L.) has a wide range ofbiological activities, such as anti-oxidativeproperties, anti-bacterial, hypoglycemic, anti-hypertriglyceridemic, anti-inflammatory, anti-fungal, anti-viral, astringent, and cytotoxic effects. This study was designed to examine the anti-hypertriglyceridemic effect of sage methanolicextract in rats.

Methods: Oral administration of sage extract (750 mg/kg body weight) and total plasma triglycerides was evaluated combined with in vitro analysis.

Results: Oral administration of 750 mg/kg of the sage extract exhibited a significant reduction in total plasma triglycerides. Also, the extract shows significant anti-lipase activity with IC50: 100.9 µg/ml, Orlistat was used as reference and showed similar anti-hypertriglyceridemic effect.

Conclusions: It is concluded that *S.triloba* has anti-hypertriglyceridemic activity andextracts from this plant show a dose-dependent activity which is comparable to the standard, anti-hypertriglyceridemic drug Orlistat.

1. Introduction



Figure 1: *Salvia triloba* Lamiaceae

Family : Lamiaceae alt. Labiatae

Genus :*Salvia*

Species :*S. triloba*

Synonyms :
Salvia libanotica

Salvia fruticosa

Salvia cypria

Salvia lobryana

Since ancient ages, people have learned to use different plants for many purposes including medicine as they were the only source avilable. Among plants widely used are many species of salvia, especially *Salvia triloba*.

1.1 The genus *Salvia*

Salvia, the largest genus in the Lamiaceae (L) family includes more than 900 species spread all around the world (Ahmadi and Mirza, 1999).The genus name *Salvia* L. is derived from the Latin *salvare* meaning 'to heal or to be safe and unharmed' referring to the medicinal properties of some of the species (Blumenthal et al., 2000).

It has been used for centuries, especially by the Chinese to promote longevity and in Romance ceremonies as a sacred herb. This name was translated to *sauge* (sage) in French and *sawge* in Old English (Codd 1985; Paton 1991; Gali-Muhtasib et al., 2000). The center of origin of the genus is speculated to be Afghanistan and central asia (Paton, 1991).

Salvia species are easily recognized by their square stems and opposite, simple pairs of leaves that are usually velvety or hairy. Leaves are often rugose, entire, toothed and lobed. Flowers are clustered in racemes, spike-like racemes. The spikes and panicles are usually large and brightly coloured, depending on the species. Flowers and stems are key diagnostic characteristics for identification of the genus (Hedge 1974; Codd, 1985).

1.1.1*Salvia triloba*

Salvia triloba is aromatic perennial herb belonging to the Labiatae family and it is endemic in the eastern Mediterranean and grows widely in Jordan, it is known as "Maryamiyah" in many arab countries like Jordan, Palestine, and Egypt (Al-Junaidy,1993; Ramadan A, 1994).

It has been widely known in the eastern Mediterranean culture although little attention has been paid to the cultivation and traditional uses of this species, this provoked question about its origin and spreed in addition to its traditional use (Rivera et al. 1994).

Salvia triloba is the generic name for sage, a strongly aromatic shrub native to the Mediterranean basin(Mouterde, 1970; Bellomaria et al., 1992; Rivera et al.,1994).

It grows widely on rocky limestone slopes at altitudes of 100 up to 900 m, flowering from February to June (Nehme, 1978).

The leaves of the plant are trilobed, with a large terminal leaflet and one pair of smaller lateral ones, wrinkled, crenulate and tomentose especially on the lower side, and green to gray in color(Meikle, 1985; Nehme, 1978).

This shrub, formerly known as *Salvia triloba* L., It is clearly different from *S.officinalis* because of its trifoliate leaves and is thus commonly known as three-lobed sage (Rivera et al.,1994). As mentioned earlier, *Salvia triloba* is endemic to the east Mediterranean and geographically distributed in Lebanon, Syria, Palestine, Crete, Cyprus, Turkey, Greece and the South of Italy and Sicily (Mouterde, 1970)

The chemical composition of its essential oil can vary depending on the season (Oil yield is maximal in the post-flowering period when the weather is dry in the Mediterranean region) (Papageorgiou et al. 2008,Bellomaria et al., 1992),the genetic diversity within the species (Bellomaria et al. 1992; Pitarokili et al. 2003; Skoula et al. 2000),the part of the plant used (leaves giving the maximum yield because of the glandular hairs present in Leaves) (Bellomaria et al 1992; Arnold and Bellomaria 1993).

1.1.2Taxonomy

Many of names of the same plant from the eastern Mediterranean are considered as synonyms of Salvia fruticosa (Greuter et al 1986).

This plantcharacterized by the variation in leaf which depends on geographical area.The plant is either three lobed leaves (*S.triloba* L.), or very small leaves (*S.libanotica*;*S.cypria*) (Mouterde 1970; Meikle 1985)

Adding to that, Carl Linnaeus gives the plant name *Salvia triloba* in 1781, while the same plant was named before by Philip Miller in 1768 as *Salvia fruticosa* (so, *S.fruticosa* is the former name know)(Clebsch, Betsy; Carol D. Barner 2003)

1.1.3 The constituentsof *S.triloba*

The odor of *S.triloba* essential oil is characteristicallycamphor-like and the taste is very bitter. Therefractive index and the specific gravity of the oilextract at 22°C are 1.46 and 0.93, respectively(Hilan et al., 1997). The oil extract includes hydrocarbons,alcohols, acids, esters, aldehydes, ketones,phenols, phenol esters, lactones, and variousnitrogen and sulfur organic compounds (Gennaro,1990; Waterman, 1993). The hydrocarbonsof chief importance are themonoterpenes(such as α- and β-pinene and myrcene), terpenes and thesesquiterpenes (Brieskorn, 1991; Geunther, 1949; Gennaro, 1990).The important alcohols in sage oil include borneol(cyclic), linalool (acyclic), and terpineol.Thujone and camphor are two important ketonespresent in the oil extracts of *S. triloba*. Oxides such as 1,8-cineole and esters such aslinalylacetate also occur in sage oil (Gennaro,1990).

Thequality and quantity of the essential oils extractedfrom *S.triloba* vary greatly with the part of theplant used (Bellomaria et al., 1992; Arnold andBellomaria, 1993) with leaves giving the maximumyield because of the glandular hairs present inleaves.

Oil yield is maximal in the post-floweringperiod when the weather is dry in the Mediterraneanregion (Bellomaria et al., 1992). Generallya long dry season results in a higher oil yield(Pitarevic et al., 1985). Furthermore, the contentof β-thujone and borneol is higher in the fullblooming stage (Verzar-Petri et al., 1985).

Rhyu characterized the essential oil composition ofsages from various origins by gas chromatography (GC) and found 1,8-cineole,α-thujone,β –thujone, and camphor to be the majorcomponents of *S.triloba* (Rhyu, 1979).Similarly,the major components of

the essential oil of*S.triloba* from Cyprus were found to be camphorand 1,8-cineole (Bellomaria et al., 1992).

According to the classificationof Cabo et al. (Cabo et al., 1987), *Salvia*species belong to one of three groups dependingon their essential oil composition:

- Group I: species high in α-thujone and β –thujone.
- Group II: those with a preponderance of linalool and linalyl acetate
- Group III: those high in 1,8-cineole and camphor

The essential oilcomposition of *S.triloba* from Lebanon wasstudied recently (Hilan et al., 1997) and found tovary slightly according to the time and site of collection. The oil yield of this plantvaried depending on the locationand season, with the greatest yield obtainedduring the post-flowering period (Hilan et al.,1997). The major component of the oil extractwas found to be 1,8-cineole and camphor (i.e. inGroup III according to Cabo's classification)

Sage also contains non-volatile components such as Caffeic acid and rosmarinic acid (Kintzios et al., 1999; Melpomeni Skoula, Jamel E. Abbes, Christopher B. Johnson, 2000), which has numerous proven antiviral, antibacterial and anti-inflammatory properties (Parnham and Kesselring, 1985).

In addition, the pentacyclic triterpenes oleanolic acid (OA) and ursolic acid (UA) in *S.triloba* are attractive due to their demonstrated biological effects like anti-inflammation, hepatoprotection, antihyperglycemia, antitumor promotion (Brieskorn et al., 1961; Liu, 1995), anti-lipase and Lipolytic activities (Junghyun Kim, Dae Sik Jang, Hyojun Kim, and Jin Sook Kim 2009).

Also, flavonoids and diterpenes (carnosol) can be found in plant material (Ulubelen et al., 1988). It has anti-oxidant activity,anti-inflammatory, lowering the risk of strokes and neurodegenerative diseases like Alzheimer's disease and amyotrophic lateral sclerosis(Burnham Institute for Medical Research 2007). Carnosol is also a promising cancer chemoprevention and anti-cancer agent (Johnson J.J. 2011).

See Table1 which summarizes some volatile oil constituent of *S.triloba*

Table 1 some volatile oil constituents:(Melpomeni Skoula, Jamel E. Abbes, Christopher B. Johnson, 2000)

Compound	
2-*β*-pinene*trans*-sabinene hydrate	
Tricycline*α*-thujone	
α-thujene*β*-thujone	
α-pinene	Camphore
Camphene	Borneol
β-pinene*cis*-pinocamphor	
Mentha-2,8-diene	Terpinen-4-ol
Octenone*α*-terpineol	
β-myrcene	Isobornyl acetae
α-phellandrene*α*-terpinyl acetate	
α-terpinene*β*-caryophyllene	
p-cymene	Aromandrene
Limonene*α*-humulene	
1,8-cineole*γ*-murolene	
γ-terpinene	Curcumene
cis-sabinene hydrate	Viridiflorene
α-terpinolene	

1.1.4 Uses of *Salvia triloba*

Since ancient times, *Salvia triloba* were used in folk. Nowadays, it is used also commercially as a spice to flavor meats such as pork, sausage and poultry (Parry, 1969; Stahl, 1973; Rosengarten, 1973; Morton, 1976).

In fact, most of the imported sage in the United States is *S.triloba* (50–95%) rather than *S.officinalis* (5–50%), although most articles on herbs describe *S.officinalis* as the commercial herb (Tucker et al., 1980).

The essential oils and water extracts of the plant are either internally used as infusions or are inhaled in steam baths.The common notion among many people who heavily use this plant in traditional medicine was that 'if it does not benefit, it would not harm' and maybe none was aware of any side effects or toxicity symptoms associated with the use of this plant.

Herbalists of Lebanon, Syria, and Jordan consider this species as a universal drug. The plant is sold in the market; the leaves are boiled as a tea for the relief of headaches, stomachaches, abdominal pain and many other disorders. The Palestinians of Israel also use this plant for the relief of stomachache, indigestion and the treatment of heart disorders (Palevitch et al., 1986). In Jordan, the plant is used for the treatment of ulcer pains and indigestion (Karim and Quraan, 1986). In Turkey, this species is used for kidney and gall bladder stones and for the relief of colds, coughs and influenza (Baser et al., 1986).

This plant as well as *S.officinalis* are very popular even among the European herbalists. As long as 400 years ago, English herbalists reported that *Salvia spp.* was good gram negative bacteria (Hilan et al., 1997).

Several investigators have also shown strong antibacterial activities associated with sage and itscomponents (Shelef et al., 1990; Evert-Ting and Deibel, 1992; Hefnawy et al., 1993; Pattnaik et al.,1997).

The essential oils of *Salvia triloba* exhibited remarkable bacteriostatic and bactericidal activity against Bacillus cereus, *Bacillus megatherium, Bacillus subtilis, Aeromonas hydrophila, Aeromonas sobria, and Klebsiella oxytoca,*and *Staphylococcus aureus* (Ana Paula Longaray Delamare et al., 2007).

Also, *S.triloba* was effective against *Pseudomonas syringae* van Hall and *Erwinia herbicola*, these bacteria cause extensive damage in agriculture(K.Karamanoli,D. Vokou,U. Menkissoglu).

In 1984, Todorov et al. conducted a study on the effect of *S.triloba* extracts onblood pressure. The sage extract was found to possess hypotensive and spasmolytic actions by lowering the blood pressure in cats and inhibiting smooth muscle contractions

induced by acetylcholine, histamine and serotonin; the extract was also found to prolong hexobarbital sleep (Todorov et al., 1984).

In addition, the essential oil extract of this plant has been found to block the activity of an enzyme linked to alzheimer'sdisease (Reuters, 1997). By blocking the enzyme, the oil extract inhibits the breakdown of acetylcholine, a chemical messenger in the brain suggesting that it may be a potentially useful drug forthe treatment of this disease.

Sage has been proven to have antioxidant (Dapkevicius et al.,1998) and anti-inflammatory properties (Schilcher, 1985), which could partly explain the reasons why this plant is so beneficial in the treatment of many human diseases.

Antioxidant activities of the seed oils of Salvia have been investigated and was found that the fatty acid components of the seed oils of these plants (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidic acids) were responsible for the antioxidant activity (R.Castro-Martinez, D. E. Pratt,1985).

Anti-implantation, antifertility and reproductive toxicity of *Salvia triloba* leaves have been investigated in male and female rats.The ingestion of *S.triloba* extract did not cause pregnancy failure. However, it reduced the number of viablefetuses and increased the number of resorptions in the pregnant rats. Digestion of *S.triloba*by adult male rats had no effect onthe number of females impregnated by these males. However, the number ofimplantations and viable fetuses werereduced in females impregnated by males , whereasthe number of resorptions was increased in females impregnated by males.On the other hand, the prenatal exposure of male and female rat offspring extract of *S.triloba*had no effects on the timing of testicular descent and vaginal opening, respectively.It would appear from these experiments that the ingestion of *S.triloba*may produce adverse effects on the fertilityof male and female rats(A. Elbetieha,M.H. Al-Hamood, A. Alkofahi, H. Bataineh, 1998)

*S.triloba*is reported to have anti-proliferative and cytotoxic activity. This activity was investigated to the adenocarcinoma of breast cell line,and showed anti-proliferative activity to adenocarcinoma of breast cell line. Althoughit does not demonstrate cytotoxic activity(Jelnar Z. Al-Kalaldeh, Rana Abu-Dahab, Fatma U. Afifi, 2010)

1.1.4.1 Internal use:

Parts used are Leaves either essentialoil or water extract:

1. Brain: improves memory (Grolier, 1992), tranquilizer, sedative (Laude et al., 1994; Fragaki, 1969; Havakis,1978), treats depression (Kbayssi, 1993), alzheimer's disease (Reuters, 1997)
2. Blood: increases circulation, treats hyperglycemia (Karim and Quraan, 1986),has spasmolytic and hypoglycemic properties (Perfumi et al., 1991), hypertension (Fragaki, 1969; Havakis,1978) blood depurative (Karim and Quraan, 1986)
3. Digestive: astringent (Laude et al., 1994; Fragaki, 1969; Havakis,1978), diarrhea (Fragaki, 1969; Havakis,1978), carminative, treats stomach ache, abdominal pain, ulcers, menstrual pain, liver pain and gases (Karim and Quraan, 1986; E. Sezik and E. Yesilada 1999)
4. Urinary: treats kidney, gall bladder stones and sand (Baser et al., 1986), diuretic (Laude et al., 1994; Ulubelen A., 1990; Hasan MG, Al-Hazimi X, Maiana GA. J, 1986),
5. Reproductive: anti-implantation, anti-fertility and reproductive toxicity(A. Elbetieha,M.H. Al-Hamood, A. Alkofahi, H. Bataineh, 1998)
6. Other: promotes longevity (Reuters, 1997), treats asthma (Kbayssi,1993),colds, influenza, coughs, expectorant, gingivitis, toothache, throat ache and rheumatic pain (Baser et al., 1986; E. Sezik and E. Yesilada, 1999; Fragaki, 1969; Havakis,1978), antifungal activity (Abou-Jawdah et al., 2002; Adam et al. 1998; Ali-Shtayeh and Abu Ghdeib, 1999; Daferera et al., 2000; Pitarokili et al. 2003; Sokovic et al.,2002),anti-inflammatory,

antimicrobial, antiviral and cytotoxic activities (Jelnar Z. Al-Kalaldeh, Rana Abu-Dahab, Fatma U. Afifi, 2010; Cardil V, Russo A, Formisano C, Rigano D, Senatore F, Apostolides Arnold N. et al, 2009; Liu X, Zu Y, Fu Y, Tao L, Gu C,WangW. et al., 2009; Kamatou G 2008; Shan B, Cai YZ, 2007; Kaileh et al., 2007; Longaray Delamare et al., 2007; Sivropoulou et al., 1997; Ahmad S, Kapadia Z, Badar Y. Fitoterapia, 1994; Darias V, Bravo L, Rabanal R, Sanchez-Mateo CC, Martin-Herrera DA., 1990),anti-oxidative properties (Exarchou et al., 2002; Matsingou et al., 2003; Ozcan 2003; Papageorgiou et al., 2008; Pizzale et al., 2002; Parnham and Kesselring, 1985) and antipyretic (Hasan MG, Al-Hazimi X, Maiana GA. J, 1986)

1.1.4.2 External use

Parts used are Leaves

1. Sore throat (Laude et al., 1994), mouth infections, sore, gum bleeding (Hilan et al., 1997),gingivitis, toothache (Fragaki, 1969; Havakis,1978)
2. Eyewash : Reduce inflammation (Grolier, 1992)
3. Essential oil : Antiseptic, antiperspirant (Valnet, 1990; Hasan MG, Al-Hazimi X, Maiana GA. J 1986)
4. Steam bath inhalations : Coughs, colds, respiratory problems, and rheumatism (E. Sezik and E. Yesilada 1999)
5. Fumigation : Insect repellent (Schilcher, 1985; Harborne, 1997; Vokou, 1992)

7. Spices : Food additive, antioxidant (Dapkevicius et al., 1998, R. Castro-Martinez, D. E. Pratt)
8. herbal tea (A. Bayrak and A. Akgul, 1987)
9. Source of essential oils (A. Bayrak and A. Akgul, 1987)
10. In perfumery.
11. Salvia specieshave been reportedly used in folk medicine for wound healing.

1.1.5 Side effect

Since the sage plant is widely used in traditionalmedicine, any irrational use could lead to manycomplications due to the establishedtoxicity of the essential oil components of thisplant (Millet et al., 1979, 1981; Geller et al., 1984;Hooser, 1990; Leushner, 1997).

Such toxicity hasbeen mainly attributed to the thujone and camphorcomponents of the oil (Millet et al., 1981) which have side effects such as nausea, mental confusion, coma,respiratory failure (Budavari 1989),epileptic convulsions, feeling of warmth, delirium and depression of the central nervous system (Laude et al., 1994),weak local analgesic and rubefacient,reproductive toxicity in rats (Leushner 1997),liver damage (Schilcher, 1985).

The oil extract has also been shown to possessinflammatory properties, which are mainly due tothe cineole component (Santos and Rao, 1997).Other effects of the oils include hypoglycemic andspasmolytic effects (Perfumi et al., 1991), epilepticreactions (Arnold, 1988; Kbayssi, 1993), loss ofequilibrium, tachycardia, and other problems relatedto the nervous system (Elisabetsky et al.,1995).

A case of intoxication was also mentionedwhereby the ingestion of three tablets of sageleaves per day by a 22-year-old young man resultedin epileptic convulsions, muscle cramps andrespiratory disorders (European Pharmacopoeia 1971).

Furthermore, it was stated by Valnet(1990) that this plant should not be consumedwhile breast-feeding because of its harmful effectson infants. Review

published bySchilcher (1985) describing the effects and sideeffects of essential oils from a variety of plantsand emphasize on the fact that these toxic effects areonly encountered due to the misuse of the plant.

In conclusion, the essential oils of *S.triloba* should be handled withcaution. In fact, theEuropean Pharmacopoeia recommends 4–6 g ofdried leaves of *S.officinalis* L. per day in terms ofinfusion (Reynolds, 1996).

1.2 Obesity

Obesity is defined as a condition associated with the accumulation of excessive body fat resulting from chronic imbalance of energy whereby the intake of energy exceeds expenditure. Excess body fat predisposes an individual to chronic diseases, such as diabetes,dyslipidemia, arterial hypertension, cardiovascular disease (CVD), diseases of the gall bladder, cancer, asthma, arthritis, osteoporosis, and Hyperuricemia(Eleonora Dal Grande 2009, Marx 2003).

Numerous studies examining the association between overweight, obesity and chronic conditions (such as, diabetes, asthma, CVD, arthritis, osteoporosis) infer that reducing overweight and obesity will decrease the prevalence of these chronic conditions (Appleton SL, et al 2006, Stanley AH et al., 2005; March L, Bagga H 2004;WHO/FAO Joint Consultation 2003; Wilson PW, et al.,2002;Lorentzen MS, Vestbo J, Lange P 2001;Mokdad AH et al., 2001;Felson DT et al., 2000, Manson JE et al., 1990).Obesity is a chronic disease consisting of the increase in body fat stores. Therefore, ideally, the definition of obesity should consider the amount of body fat.

The Neel's thrifty gene hypothesis (Neel JV. 1962) suggests that the human species has a very efficient capability to store energy as fat. In fact, this is a defence mechanism that has facilitated the preservation of the species when our ancestor's life conditions were as hard as they were during the early years of the presence of human life on Earth. Butwhen the big revolution occurred (industrialization)during the last six or seven decades, we as humans have been developing machines that could help us save energy. The result is that nowadays our daily lives require minimal energy expenditure. From that point, the genetically determined energy storing capacity turns against us making us becoming obese, with type 2 diabetes, hypertensive, etc. This is

how, what was initially a positive genetic trait, becomes a deleterious characteristic, leading to what the World Health Organization (WHO) has qualified as the 21st Century epidemic: obesity (WHO Consultation on Obesity 2000).

1.2.1 Measuring body fat

Measuring body fat requires quite sophisticated methods that make population-based measure of body fat almost impossible to perform. Consequently, there are not precisely defined normal values of body fat. Thus, for practical reasons, obesity is measured by:

1. Body Mass Index (BMI)
2. The waist circumference
3. The waist-to-hip ratio

1.2.1.1Body Mass Index (BMI)

BMI=Weight (kg)/ $(\text{height})^2$ (m)

BMI highly correlates with total body fat and is very useful for epidemiological purposes. Based on the BMI and on its relationship with death from all causes, the WHOhas established different cut-off points enabling the classification of obesity (Ta b l e 2).This WHO classification of obesity is universally accepted as a measure of obesity in adults (WHO Consultation on Obesity 2000).

Ta b l e 2: WHO Classification of adult obesity

Classification of adult obesity		
Classification BMI (kg/m^2) Risk of comorbidities		
Normal range	18.5–24.9	Average
Overweight	≥ 25.0	
Pre-obesity	25.0–29.9	Increased
Obesity class I	30.00–34.9	Moderate
Obesity class II	35.00–39.9	Severe
Obesity class III	≥ 40.0	Very severe

1.2.1.2The waist circumference

Regarding the health risks related to obesity, we have to take into account not only the magnitude of obesity but also, and perhaps more relevant, the body fat distribution. There are two main types of obesity regarding the fat distribution pattern: android or central type obesity with the majority of fat depots located in theabdominal area, both subcutaneous and visceral, and gynoid or peripheral obesity in which the fat depots are mainly located subcutaneously in the lower body (hips and lower extremities). The difference between both types is fundamental, because themetabolic and cardiovascular complications of obesity are almost exclusively related to visceral fat depots.

Because of its high correlation with visceral adipose tissue(Despres JP 2001) a measure as simple as the waist circumference is a good way to assess cardiovascular risk. According to McLean (Lean MEJ, Han TS and Morrison CE 1995), waist circumference cut off points can be established both for men and women to assess cardiovascular risk.

A waist over 88 cm for women and above 102 cm for men indicates a high cardiovascular risk and the need for action.

1.2.1.3The waist-to-hip ratio

The waist-to-hip ratio (WHR) is still a useful measure to be consideredin obesity evaluation.

1.2.2 EPIDEMIOLOGY OF OBESITY

Due to its high worldwide prevalence, obesity is currently the most common metabolic disease in the world. The WHO estimates that more than one billion people are overweight and, of these, 300 million can be considered as obese, with a BMI above 30 kg/m^2 according its own BMI based classification of obesity. Actually, there is a great concern because the global figures of obesity are progressively increasing from an estimate of 200 million people affected in 1995 to the current 300 million, which is a 50% increase in only seven years. It is estimated that if no action is taken against this, these figures could double in 20 years. Moreover, this increase in obesity prevalence is a global trend, not only confined to affluent societies but also seen in emerging countries such as China(WHO Consultation on Obesity, 2000; Wu Y, Zhou B, Tao S et al., 2002) and Argentina in which the prevalence of overweight and obesity was 52 and 17%, respectively (Laura R. Aballay et al., 2009).

In fact, Prevalence estimates for overweight and obesity have increased more in many developing countries than the US and European countries in recent decades (Filozof Cet al., 2001).As well known,Obesity can be found in adults and children.

In Finland during 1992-1993, 14% of all 7-year-old boys and 18% of girls were found to be overweight or obese among a sample of 8416 children(Laitinen J et al., 2005).

Ten years later, in another sample of 2646 children, 16% of the boys in the same age group and 20% of the girls were overweigh(Rintanen H 2005).The increasing prevalence of overweightchildren is of concern because overweight children are at increased risk for social stigmatization, adult obesity and chronic disease (Ebbeling CB, 2002).

In Japan there is also a growing tendency in childhood obesity prevalence. However, this increase seems to be more important in small rural towns, with the girls living in metropolitan areas not being a part of this phenomenon. These differences between trends in rural and urban areas are important when consideringobesity prevention or intervention policies(Matsushita Y et al., 2004).

Also, childhood obesity is now endemic in Australia (Booth ML, Wake M, Armstrong T, Chey T, Hesketh K, Mathur S, 2001).Epidemiological studies comparing current and previous data clearly demonstrate this rising trend.

In fact, the prevalence of class II and III obesity increased significantly between 1991 and 2006 in Australia (Natasha J. Howard, Anne W. Taylor, Tiffany K. Gill, Catherine R. Chittleborough 2008).

Moreover, in the U.S.A., the prevalence of adult obesity (BMI$\geq$30 kg/m^2) hasdoubled from 1986 to 2000, from 10% of the adult population to a current 20%. Also,during the same period of time, the prevalence of severe obesity quadrupled from 0.5to 2% of the adult population (Sturm R. 2003).

Figure 2 is an attempt to summarize the current figures about the prevalence ofobesity in some countries that periodically undertake population-based health surveys.

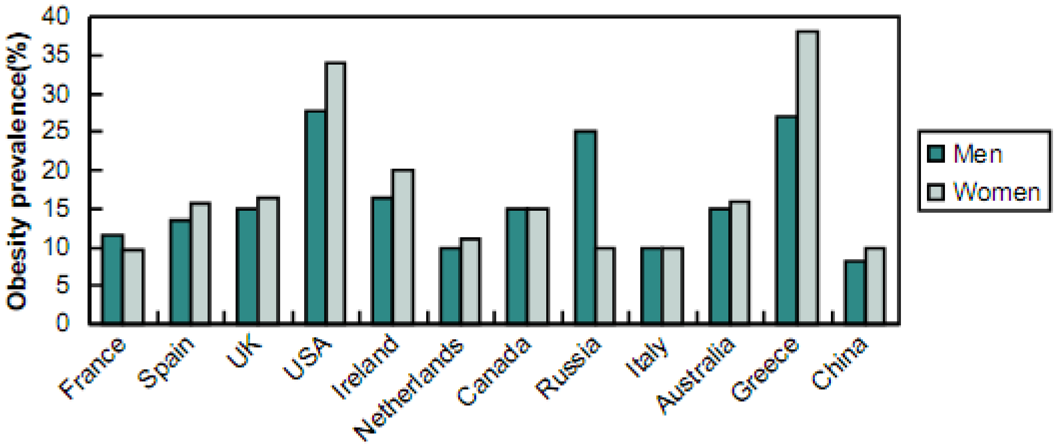


Figure 2.Prevalence of obesity in some countries undergoing epidemiological surveys. (Modified from: International Obesity Task Force Report 2002. Accessible from: www.iotf.org.)

Reports suggest that our current lifestyle of processed foods and decreased physical activity may shorten the modern human life span.

The high incidence of obesity, its impact on life expectancy in adults and children, and the lack of safe therapeutic agents, have fuelled an increase in anti-obesity drug-related research. Many Scientists reported that life expectancy in adults and children are now reduced due to obesity (Olshansky *et al*., 2005).

1.2.3 HEALTH CONSEQUENCES OF OBESITY

There are many health and non-health consequences of obesity. Non-health consequences of obesity are psychological factors that include anger, anxiety, and depression (Wing RR et al., 19991; Wellman NS, Friedberg B, 2002).

Laura R. Aballay et al study the non-health consequences on overweight and obese population and revealed that in Córdoba city (Argentina) overweight was positively associated with gender (men), decreasing socio-economic status, married and divorced status and negatively with increasing levels of physical activity (Laura R. Aballay et al 2009).

In a prospective study done on a cohort of more than one million adults in theUnited States, Calle et al, found in healthy people who had never smoked, a relativerisk of death of 2.58 and 2.00 for men and women, respectively, among those with thehighest BMI as compared with those with BMI of 23.5 to 24.9(Calle EE, ThunMJ, Petrelli JM et al., 1999).

Data from theFramingham Hearth Studyshow a significant reduction in life expectancy amongnonsmoking obese people in comparison with nonsmokers at normal BMIrange (18.5 - 24.9 kg/m^2). Such reduction accounts for 7.1 years for females and 5.9years for males(Peeters A, Barendregt JJ,Willekens F et al 2003).Anyway, it seems that the effect of BMI on mortality is age-dependent, so the relative risk associated with greater BMI declines with age (Stevens J, Cai J, Pamuk ER et al 1998).

Besides being a shortening life factor, obesity and precisely central type obesity, is commonly associated with many other diseases mainly in the cardiovascular and

metabolic area. Thus, central type obesity is often associated with high blood pressure, ischemic heart disease, stroke, type 2 diabetes and dyslipidemia(Eleonora Dal Grande et al., 2009; Grundy SM et al., 2004;Marx, 2003; Hubert HB et al.,1983)

Obesity is well established as an independent predictor of myocardial infarction (MI) across a broad range of ethnic groups (Yusuf S, Hawken S et al., 2005). Furthermore, associations have been established with obesity and heart failure (Kenchaiah S et al 2002), both arterial (Shimokawa H, 1999; Stamler R et al, 1978)

and pulmonary (Amad KH et al., 1965) hypertension, stroke (Shinton R et al., 1991) and atrial fibrillation (Gami AS et al., 2007).

Eleonora Dal Grande studies the contribution of overweight and obesity to five chronicconditions using the population attributable risk (PAR) statistic. For the period 2004-2006 (n = 15,261), 19.0% of South Australians aged 18 years and over were obese, and 36.0% were overweight. The PAR of overweight and obesity was highest among those with diabetes (17.3% and 28.4%, respectively). After adjusting demographic and risk factors, overweight and obesity had a significantly high PAR for diabetes (11.3% and 23.1%), asthma (4.0% and 10.3%) and arthritis (6.7% and 8.1%). The unadjusted PAR of overweight and obesity for CVD was significant (10.8% and 11.7%) but was reduced when controlled for other factors. The PAR of overweight and obesity was significant for osteoporosis but the association was protective (Eleonora Dal Grande 2009).

These effects can be explained as obesity (especially central obesity) can cause endothelial dysfunction which may be a key mechanistic link between obesity and associated increase in cardiovascular events (Yusuf S et al., 2005; Knudson JDet al., 2005; Mombouli J-V, Vanhoutte PM, 1999).

Endothelial dysfunction, evaluated both in the peripheral and coronary circulation is associated with adverse cardiovascular events, particularly at the microvascular level (Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T 2001).

The vascular endothelium is now known to have a pivotal role in the maintenance of vascular homeostasis including effects on vasomotor and platelet function, thrombus formation and fibrinolysis, cell growth and inflammation largely through bioavailability of endothelial-derived nitric oxide (Schachinger V et al., 2000; Suwaidi JA et al., 2000;Mombouli J-V, Vanhoutte PM, 1999; Steinberg HO et al., 1996; Creager MA et al., 1990).

Altered endothelial function (endothelial dysfunction), occurs in the setting of atherosclerosis, hypertension, hypercholesterolemia and diabetes mellitus.

Moreover, recent studies have shown that metabolic syndrome results from the upregulated production of offensive adipokines, including Plasminogen Activator Inhibitor-1 (PAI-1) (Shimomura Iet al., 1996), Tumor Necrosis Factor-α (TNF-α)

(Hotamisligil GS et al., 1993), Interleukin-6 (IL-6) (Yudkin JS et al., 2000; Fried SK et al., 1998), monocyte chemotactic protein-1 (MCP-1) (Sartipy P et al., 2003) and angiotensinogen (Hainault I et al., 2002), and also from the downregulated generation of defensive ones such as adiponectin (Matsuzawa Yet al., 2004) and leptin (Farooqi IS et al., 2001), all of which are derived from visceral adipose tissue.

Offensive adipokines enhance reactive oxygen species (ROS) production, resulting in augmented oxidative stress that induces systemic chronic inflammation. Indeed, a close association between oxidative stress and abdominal obesity has been demonstrated (Pou KM et al., 2007).

Apart from these metabolic and cardiovascular disturbances, obese people are frequently suffering from joint diseases and respiratory disorders such as obstructive sleep apnea (OSA) andobesity hypoventilation syndrome (OHS)(Bahar Ulubas, 2009;Saxena Yet al, 2008; Olson AL, Zwillich C, 2005).

1.2.3.1 Insulin resistance and type 2 diabetes mellitus

The relationship between obesity and insulin resistance applies to the full range of body weights.It is well known that insulin is a regulator of the adipocyte biology andadipocytes are one of the cells of the body with a higher response to insulin. Insulinfosters the differentiation of pre-adipocytes to adipocytes, stimulates lipogenesis, andinhibits lipolysis(Kahn BB and Flyer JS. 2000).

Insulin resistance is defined as the decreased effect of insulin on glucose uptake, metabolism and storage. There is a diminished insulin stimulated glucose uptake in skeletal muscle and adipose tissue, and impairment in the suppression of hepatic glucose output. These defects may be determined by impaired insulin signaling or by the downregulation of the glucose transporter, GLUT4. In relation to the signaling defects in obesity, it is important to consider that the initial signal for insulin action is theactivation of the tyrosine kinase of the insulin receptor, which determines the phosphorylation of several tyrosine residues. In muscle and adipose tissue of obese humans and rodents there is an increased expression and/or activity of several protein tyrosine phosphatases, which dephosphorylate and terminate signaling propagated through tyrosine phosphorylation (Goldstein BJ, Ahmad F, Ding W et al. 1998).

Additionally, a reduction in the expression of several insulin signaling molecules has been described in skeletal muscle in morbid obesity. In the case of GLUT4 expression, it seems to be normal in skeletal muscle of obese and diabetic subjects that defective glucose transport appears to be due to impairment of translocation, docking, or fusion of GLUT4containing vesicles with theplasma membrane (Shepherd PR and Kahn BB. 1999).

Obesity is accompanied by the production of several cytokines that decrease insulin sensitivity in liver and skeletal muscle. TNF-α has paracrine effects on adipose cells, and reduces insulin action in skeletal muscle (Hotamisligil GS. 1999).

Free fatty acids (FFA) released from the more lipolytically active intra-abdominal adipocytes, increase insulin resistance in liver and skeletal muscle through mechanisms affecting the intracellular insulin signaling cascade (Griffin ME, Marcucci MJ, Cline GW et al., 1999).On the contrary, adiponectin, a true hormone acting through receptors in insulin target tissues as skeletal muscle and liver, improves insulin sensitivity by enhancing intracellular insulin signaling (Yamauchi T, Kamon J, Waki H et al. 2001).A reduced serum adiponectin has been reported in obesity (Arita Y, Kihara S, Ouchi N et al., 1999; Weyer C, Funahashi T, Tanaka S et al., 2001).

However, other studies have shown that it is the subcutaneousadipose secretion rate of adiponectin which is reduced in obese compared with non-obese subjects, but not serum levels, which are non-significantly different from those ofnon-obese patients. The fact that insulin resistant individuals have lower values of bothadipose secretion rate and serum concentration than subjects with high insulinsensitivity would suggest that the degree of hypoadiponectinemia in obesity is related toinsulin resistance rather than body fat (Hoffstedt J, Arvidsson E, Sjolin E et al. 2004).

Although not all obese individuals develop type 2 DM, the increase in the prevalence of obesity has been associated with an increase in its prevalence (Mokdad AH, Ford ES, Bowman BA et al. 2001), and in the Nurse's Health Study overweight or obesity was the most important predictive factor (Hu FB, Manson JE, Stampfer MJ et al. 2001).

Again, central obesity is found to be a major risk factor independently of the severity of obesity (Boyko EJ, FujimotoWY, Leonetti DL and Newell-Morris L. 2000).

In the last decade there is increasing evidence suggesting a positive association between low birth weight and impaired glucose tolerance, and even type 2 DM in adulthood (Jaquet Dand Czernichow P. 2003).

Bhargavaet alhave reported, studying more than 1400 adults in India, that low body weight andthinness at one to two years of age were associated with impaired glucose toleranceand diabetes in adulthood. This low BMI was followed by an early adiposityrebound (the age after infancy when body mass starts to rise) and an accelerated weight increase afterwards (Bhargava SK, Sachdev HS, Fall CH et al. 2004).

Patients with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) are now referred to as having 'pre-diabetes', indicating the high risk of future diabetes as well as cardiovascular disease.

According to the American Diabetes Association (ADA), the IFG is defined as a fasting plasma glucose (FPG) of 100–125 mg/dl (5.6-6.9 mmol/l) and IGT as a 2-hourpost-load glucose (after 75 g of glucose) of 140–199 mg/dl (7,8–11,1 mmol/l) (American Diabetes Association 2004).

Criteria for the diagnosis of diabetes mellitus are presented in Table 3 (American Diabetes Association 2004).

Table 3: Criteria for the diagnosis of diabetes mellitus

Normoglycemia	IFG/IGT	Type 2 diabetes mellitus
FPG < 100 mg/dl (5.6 mmol/l)	IFG: FPG 100 – 125 mg/dl (5.6 – 6.9 mmol/l)	FPG ≥ 126 mg/dl (7.0 mmol/l) or
2-hours postload Glucose < 140 mg/dl (7.8 mmol/l)	IGT: 2-hours postload glucose ≥ 140 mg/dl (7.8 mmol/l) and < 200 mg/dl (11.1 mmol/l)	2-hours postload glucose ≥ 200 mg/dl (11.1 mmol/l) or
		Symptoms of diabetes plus Casual FPG ≥ 200 mg/dl (11.1 mmol/l)

1.2.3.2 Dyslipidemia

Obesityassociated dyslipidemia plays a crucial role in the development of atherosclerosis and cardiovascular disease in obese subjects, because all the elements of the dyslipidemia normally associated with this disease have been shown to be atherogenic.

Obesity, mainly of central fat distribution, is associated with increases in plasma triglycerides, and decreases in HDL cholesterol. In fact, when adjusting for BMI, patients with a higherWHR seem to have higher triglycerides levels and lower HDL cholesterol levels (Dowling HJ and Pi-Sunyer FX, 1993).

The effect of obesity on LDL cholesterol levels is not so clear. To this respect, obese young men have LDL concentrations higher than those of normal weight, but in middle age and older men, only minimal differences occurred (Denke MA, Sempos CT and Grundy SM, 1993).

Interestingly, a change in LDL composition occurs in obese individuals whose LDL particles are smaller and more dense, increasing the risk of cardiovascular disease in individuals with this kind of LDL particles than in those with large LDL particles for the same level of total cholesterol and, again, associated with a central distribution of the fat (Williams PT and Krauss RM, 1997).

The reduced diameter of these particles favours their movement through the endothelial fenestrations, and their location in the subendothelial space determining the formation of the plaque.

This obesity associated pattern of dyslipidemia (high trygliceride levels, low HDL concentrations, and dense and small LDL particles) is related to insulin resistance. The presence of abdominal obesity is more highly correlated with the metabolic risk factors than is an elevated BMI. Lipoprotein a (LPa) is known to be an independent risk factor for coronary, cerebrovascular and peripheral vascular disease when its serum concentration exceeds 30 mg/dl. Structurally LPa is very similar to LDL cholesterol and plasminogen and its levels are relatively constant throughout life. Higher than normal serum concentrations of LPa are commonly seen in central type obesity (Wassef N, Sidhom G, Zakareya el-K and Mohamed el-K, 1997).Simple variables, such as triglyceride levels and waist circumference can be used to identify

individuals with a cluster of new metabolic risk factors for cardiovascular disease, such as hyperapolipoprotein B, small dense LDL levels and hypertriglyceridemia. It is the known concept of the 'hypertriglyceridemic waist': A marker of the atherogenic metabolic triad (hyperinsulinemia, hyperapolipoprotein B, small, dense LDL) (Lemieux I, Pascot A, Couillard C et al., 2000).

The primary defect in lipid metabolism in obesity seems to be the overproduction of VLDL by the liver. In the insulin resistant state, normally present in these patients, there is a decreased ability of insulin to suppress hepatic glucose output and the release of free fatty acids (FFA) from adipose tissue. These events play an important role in hepatic VLDL production. Besides this increased synthesis, there is a decreased clearance of triglyceride rich lipoproteins in the circulation due to decreased lipoprotein lipase activity (Miyashita Y, Shirai K, Itoh Y et al., 2002).

1.2.3.3 Hyperuricemia

Hyperuricemia is frequently observed in obese subjects due to the overproduction of uric acid. Matsuura et al, reported that purine synthesis and the overproduction of uric acid could be linked to fatty acid synthesis by the liver (Matsuura F, Yamashita S, Nakamura T et al., 1998).

Also, the amelioration of insulin resistance by either a low energy diet or insulin sensitizing agents decreases the serum uric acid concentration in overweight hypertensive patients (Tsunoda S, Kamide K, Minami J and Kawano Y, 2002) which suggest that insulin resistance (frequently occurred in obese and overweight individuals) increases uric acid production.

1.2.3.4 Cardiovascular disease

Obese subjects have increased risk of cardiovascular disease (CVD), and this is true especially for the abdominal or central type obesity. Obese individuals with central fat distribution are at higher risk for heart disease than those with peripheral type of obesity. The connection between obesity and CVD is not simple (Sowers JR, 2003).

The atherosclerotic process is apparently regulated by inflammatory mechanisms (Ross R, 1999) and systemic inflammation has been linked to insulin resistance in a large number of human population studies.

1.2.3.4.1Hypertension

Among all the obesity related comorbidities, high blood pressure is, probably, the most well known. There is a huge amount of epidemiologic evidence demonstrating this relationship{both arterial (Shimokawa H, 1999;Stamler R et al., 1978) and pulmonary (Amad KH, Brennan JC, Alexander JK, 1965) hypertension}.

The Health Professionals Followup Study shows that among obese men(BMI≥30 kg/m^2) around 35% are hypertensive (Baik I, Ascherio A, Rimm EB et al., 2000).

Also, in women there is a strong relationship between obesity and hypertension. In the Nurse's Health Study it is clearly seen that, women with a BMI over 30 kg/m^2 have a four-fold increase in relative risk ofhaving high blood pressure in comparison with those with a BMI of 21 kg/m^2 (Huang Z, WilletWC, Manson JE et al., 1998).

Whereas the epidemiological evidences are overwhelming, the physiopathological mechanisms linking obesity and hypertension are not fully known.It is clear that hyperinsulinemia is not the cause of the hypertension in the obese (Feskens EJM, Toumilehto J, Stengard JH et al. 1995).

Carlson SH et al,shows that an increase in sympathetic activity and sodium retention is commonly in obese hypertensive humans, whichcould explain, at least in part, the hemodynamic disturbances (Carlson SH, Shelton J, White CR and Wyss M, 2000).

Also, impaired endotheliummediated vasorelaxation could contribute to hypertension (Shimokawa H, 1999).

Leptin, a 167 amino acid peptide hormone, might be also potentially involved in thehypertension of the obese. Nevertheless, whereas there is some evidence of its causalrole in laboratory animals, the results of the studies performed in humans aresomewhat confusing, and therefore it is not possible by any means, to ascribe to leptinany causal role in obesity hypertension (El-Atat F, Aneja A, Mcfarlane S and Sowers J, 2003).

The renin-angiotensin-aldosterone system (RAAS) probably has a key role in obesityhypertension. The RAAS seems to be hyper-activated in obesity, in spite of sodium retention. Engeli and Sharma have demonstrated direct association between plasma

leptin and angiotensin levels suggesting that adiposetissue may contribute to the plasma angiotensin level (Engeli S and Sharma AM, 2001).

In recent years it has become manifest that adipose tissue possesses a local RAASwhich is supposed to play an important role in adipose tissue functioning. For instance,angiotensin II is involved in the metabolism and differentiation of adipose tissue and inlipid storage. In fact, in central obesity, a positive relation between the waist-to-hip ratio (WHR) and the expression of angiotensinogen mRNA in the omentum has been found (Van Harmelen V, Elizalde M, Ariapart P et al., 2000).

Recently, angiotensinogen production by human cultured fat cells has been shown, though not having any relationship with the BMI (Prat-Larquemin L, Oppert JM, Clement K et al., 2004).

1.2.3.4.2Heart disease

Obesity is associated with several heart abnormalities.In a study of the American population, there was a significant increase in the prevalence of heart disease, diabetes, hypertension and hypercholesterolemia with increasing body weight in all gender, racial and socioeconomic groups (Paeratakul S, Lovejoy JC, Ryan DH, and Bray GA, 2002).

Manson JE et al found that Obesity increases developing coronary artery disease, up to 3.3-fold in American women with a BMI over 29 kg/m^2, in comparison with women with a BMI below 21 kg/m^2 (Manson JE, Willet WC, Stampfer MJ et al., 1995).

Moreover, a WHR ≥ 0.92 is associated with a 3-fold increased risk of coronary heart disease in men (Lakka HM, Lakka TA, Tuomilehto J and Salonen JT, 2002).

Again, IL-6, a cytokine produced by adipose tissue,seems to play a key role in the development of coronary heart disease associated withobesity through different metabolic, endothelial and pro-coagulant mechanisms (Yudkin JS et al., 2000).

Left ventricular hypertrophy is frequent in obesity, and is not just related to concomitant hypertension. Increases in stroke volume, cardiac output and diastolic dysfunction are seen in obese subjects without hypertension. The changes affecting the left ventricle have been related to sudden deaths in these patients. Not only the left

ventricle but also the right ventricle presents some changes. As a consequence of left ventricular dysfunction or the coexistence of OSA and/or OHS, cor pulmonale can occur. Therefore, for all these reasons heart failure in obesity is frequently biventricular.

Obesity can also be the cause of arrhythmias, and the prolonged QT interval also seen in obesity might be a predisposing factor for sudden death (Eckel RH, 1997).

Also, in patients with morbid obesity, although the cardiopulmonary capacity appears to be normal, the exercise capacity is significantly reduced, as compared to control subjects (Seres L, Lopez-Ayerbe J, Coll R et al., 2003).

1.2.3.4.3Stroke

The association between obesity and stroke remains controversial, although most studies have shown a positive relation (Shinton R, Shipley M, Rose G, 1991).

A prospective and interesting study done in 234,863 Korean men has demonstrated that BMI is a risk factor for both ischemic and hemorrhagic stroke (Song YM, Sung J, Davey Smith G. and Ebrahim S, 2004).

Other studies have underlined abdominal obesity as an independent risk factor for ischemic stroke in all race-ethnic groups, being a stronger risk factor than BMI and with a greater effect among young subjects (Suk SH, Sacco RL, Boden-Albala B et al., 2003).

Plasma leptin has been considered a risk factor for first-ever hemorrhagic but not ischemic stroke, in an independent manner of other risk markers for CVD (Soderberg S, Ahren B, Stegmayr B et al., 1999).

If we consider that most obese people have leptin resistance with high levels of plasma leptin (Considine RV et al., 1996), we could speculate that leptin might be the link between obesity and hemorrhagic stroke.

1.2.3.5Gallbladder disease

Cholelithiasis is the primary hepato-biliary pathology associated with obesity. The risk of having gallbladder disease is positively associated with the BMI. In men, this association has been reported less consistently than in women. In a population-based study, central obesity was related to gallbladder disease in both sexes (Ruhl CE and Everhart JE, 2001).

Csendes et al 69 reported in a prospective study of 125 obese patients a high frequency of gallstones (30.4%) (Csendes A, Burdiles P, Smok G. et al., 2003).

The explanation for the cholelithiasis seems to be that a higher excretion of cholesterol through the bile in obese patients, favoured by highplasma levels of cholesterol and triglycerides frequently found in obesity. This high concentration in relation to bile acids and phospholipids increases the probability of precipitation of cholesterol gallstones in the gallbladder.

1.2.3.6Locomotor system

There are functional locomotor limitations in obese individual. They have flexibility troubles and frequently pain in performing tasks at floor level such as picking things up, squatting or kneeling. They also move more slowly, and experience more exertion than the control population (Larsson UE and Mattsson E, 2001).

On the other hand, osteoarthritis and other joint problems are more frequent in obese subjects. The first National Health and Nutrition Examination Survey showedthat women with a BMI between 30–35 kg/m^2had nearly four times the risk of arthritis of the knee than women with a BMI under 25 kg/m^2 (Anderson JJ and Felson DT, 1988).

In fact, several case control studies have demonstrated a strong association between knee osteoarthritis and obesity and in the Framingham study high BMI predicted development of the disease (Felson DT, Anderson JJ, Naimark A et al., 1988).

A population-based case-control study in three districts of England has shown that a large proportion of severe knee osteoarthritis is attributable to obesity, being this risk especially high among obese subjects with other risks factors as generalizedosteoarthritis and surgery or knee injury (Coggon D, Reading I, Croft P et al., 2001).

Arthritis develops as a consequence of trauma associated with the excess of body weight in joints such as the knees and the ankles.

1.2.3.7Obesity-related kidney disease

Obesity is related also to kidney disease. The renal effects of obesity include both structural and functional changes. Both glomerulomegaly and focal segmental glomerulosclerosis have been associated with obesity, not only massive obesity but also class I and II obesity. In relation to functional adaptations, increased glomerular filtration rate, increased renal blood flow and renal hypertrophy are considered to be the most relevant. Clinically, the presentation of obesity-related glomerulopathy is nephrotic or sub-nephrotic range proteinuria and renal insufficiency in 44% of cases. In 45% of cases, biopsies show focal glomerular basement membrane thickening or focal mesangial sclerosis, as seen in early diabetic nephropathy (Kambham N, Markowitz GS, Valeri AM et al., 2001).

Indeed, an increased BMI has been associated with a potential marker of cardiovascular disease such as microalbuminuria, seen mainly in subjects with hypertension (Ribstein J, du Cailar G and Mimran A, 1995).

In a subanalysis of the PREVEND (Prevention of Renal and Vascular End Stage Disease) study, the prevalence of microalbuminuria (30–300 mg/24 hours) in men increased from 9.5% in those with normal body weight, to 18.3% in overweight subjects, and to 29.3% in individuals with obesity. These percentages in women were 6.6, 9.2 and 16%, respectively (De Jong PE, Verhave JC, Pinto-Sietsma SJ and Hillege HL, 2002).

Obesity may have similar effects as diabetes on the kidney, with an initial phase of glomerular hyperfiltration followed by microalbuminuria, with this phase followed by a progressive fall in glomerular filtration rate and a further rise in urinary

albuminexcretion with the development of proteinuria and, sometimes, end-stage renal failure (Pinto-Sietsma SJ, Janssen WM, Hillege HL et al., 2000).

This hypothesis suggesting that glomerular hyperfiltration underlies the pathogenesis of glomerulopathy in obesity is reinforced by experiments in obese Zucker rats (Maddox DA, Alavi FK, Santella RN and Zawada Jr. ET, 2002).

The exact mechanism behind the obesity-related kidney damage is not known. But hemorheologic alterations such as higher blood viscosity, hyperfibrinogenemia, andlower erythrocyte deformability values have been related to proteinuria in patients with central obesity (Solerte SB, Fioravanti M, Pezza N et al., 1997).

Hyperleptinemia and insulin resistance could be some of the other involved mechanisms, since insulin resistance induces intraglomerular hypertension as well as mesangial hypertrophy (Kambham N, Markowitz GS, Valeri AM et al., 2001).

On the other hand, the inflammatory changes in the setting of obesity could also be another of the mediators of renal disease, since higher CRP levels are also associated with a higher relative risk of impaired glomerular filtration (Stuveling EM, Hillege HL, Bakker SJ et al., 2003).

1.2.3.8Obesity and cancer

There is a growing body of evidence that obesity has also some kind of causeandeffect relationship with many types of cancers. Given the global rising trend in obesity prevalence, even a small added risk of cancer due to obesity should be a major concern.

In the U.S.A., an increase in the relative risk of dying from cancer has a clear positive association with the BMI both for men and women. Assigning the risk 1 to people with a normal BMI (18.5–24.9), those with obesity class II have a 1.3-fold relative risk, whereas those with a BMI ≥40 kg/m^2 have a relative risk higher than 1.5 (Adami HO and Trichopoulos D, 2003).

The study by Calle et al is a prospective cohort investigation done on a very large population sample (more than 900,000 U.S.A. adults), with a follow up of 16 years. The authors examined the relation between the BMI in 1982 and the risk of death from all cancers and from different types of cancer, calculating also the proportion of all deaths from cancer that can be attributable to overweight and obesity in the U.S.A. The results of the study clearly show that being obese increases the risk of death from all types of cancer. Regarding the mortality from specific types of cancer, people in the highest category of BMI have a significantly higher risk of dying from almost all types of cancer than those in the normal BMI range (18.5–24.9 kg/m2). For women, the risk of death from endometrial cancer is 6.25-fold higher in obese women in comparison with those with a normal BMI. Obese men have a relative risk of death from liver cancer of 4.52 compared with non-obese ones. The influence of fat distributionwas, unfortunately, not analysed in this study (Calle EE, Rodriguez C,Walker-Thurmond K and Thun MJ, 2003).

A similar population-based study done in Canada also showed a strong direct association between obesity and cancer. The authors found that, overall, obesity accounted for 7.7% of all cancers in Canada, providing further support for the positive association of obesity with cancers of the kidney, colon, rectum, breast (in postmenopausal women), ovary, pancreas and prostate. The occurrence of endometrial cancer was not reported in this study (Pan SY, Johnson KC, Ugnat AM et al., 2004). In women, the relationship between BMI and breast cancer seems to be age dependent. Thus, premenopausal obese women might have a decreased risk of breastcancer as compared with those premenopausal women within the normal range BMI (Tornberg SA and Carstensen JM, 1994).

Thus, there is a huge amount of epidemiological evidence about the relationship of obesitywith cancer. Less clear are themechanisms throughwhich obesity predisposes to cancer.

One of the factors commonly may involve is estrogens: obesity is commonly associated with elevated free circulating estrogens level due to increased production from adipose tissue (Nelson LR, Bulun SE, 2001).

Recently Moore et al, in a cohort of patients from the Framingham Study, found that the degree of central obesity, measured by waist circumference, is a stronger predictor of colon cancer risk than is BMI (More LL, Bradlee ML, Singer MR et al., 2004).

Central obesity is associated withhigh levels of insulin-like growth factor 1 (IGF-1) which is capable of inhibiting apoptosisand stimulates cell proliferation. IGF-1 is associated with diverse types of cancer(breast, ovarian, colorectal, lung, prostate and bladder) (Friedrich MJ, 2003).

Thus, central fat distribution could be involved in the increased cancer risk of obese people. Adiponectin, an hormone produced by adipose cells with insulin sensitizing properties, has been shown to be inversely related to the degree of obesity and insulin resistance. Adiponectin is also involved in cancer risk. The group of Trichopoulos recently reported an inverse association between plasma adiponectin levels and the risk of endometrial (Dal Maso L, Augustin LS, Karalis A et al., 2004) and breast (Mantzoros C, Petridou E, Dessypris N et al., 2004) cancers, the latter being found only in postmenopausal women.

Some genes could also be involved in the association of obesity and cancers. This is the case of the peroxisome proliferator activated receptor-γ (PPAR-γ) which might be involved in prostate cancer (Paltoo D, Woodson K, Taylor P et al., 2003).

1.2.3.9Respiratory disease

Two of the most common respiratory disturbances found in obesity are: obstructive sleep apnea (OSA) and obesity hypoventilation syndrome (OHS). In severe cases, both diseases can constitute a real threat for the patient's life.

1.2.3.9.1 Obstructive sleep apnea (OSA)

The prevalence of OSA in the general population is highly variable, from 25 to 58% of men and 10 to 37% of women, depending on the race and the geographic area studied (Young T, Palta M, Dempsey J et al., 1993).

But symptomatic OSA, with daytime somnolence, is supposed to affect only4% and 2% of middle age men and women respectively (Young T, Shahar E, Nieto FJ et al., 2002).

This means that the majority ofpatients suffering from OSA is not diagnosed and therefore not treated, which ispotentially harmful.

The association of obesity with OSA is strong, it is known that around 70% of individuals with OSA are obese and that the prevalence of OSA among obese people is approximately 40% (Resta O, Foschino-Barbaro MP, Legari G et al., 2001).

In grade III obesity, OSA is almost the rule (Valencia-Flores M, Orea A, Castano VA et al., 2000).

The events characterizing OSA are the apneas caused by the occlusion of the upper airway. The immediate consequence of the apnea is a decrease in the oxygen concentration which is responsible of the arousal, bringing the patient to a lighter

sleepstate, making it possible to restore the airflow and, consequently, return the oxygen concentration to normal levels. Nevertheless the arousal is transient and the sequence of events is repeated again and again during the sleep time (AASM:American Academy of Sleep Medicine 2001).

The severity of OSA is defined by the apnea-hypopnea index (AHI) which is the number of apnea or hypopnea episodes per hour. Currently, it is considered that anAHI above 5 is diagnostic of OSA and severe OSA is diagnosed when the AHI is greater than 20 (Gami AS, Caples SM and Somers VK, 2003).

The cause of OSA in obesity is related to the narrowing of the upper airway due to an extrinsic soft tissue enlargement because of fat deposits in the oropharyngeal area.

1.2.3.9.2 Obesity-hypoventilation syndrome

The other respiratory disturbance associated with obesity is the poorly known obesity hypoventilation syndrome (OHS) (also known as **Pickwickian syndrome**) is a condition in which severely overweight people fail to breathe rapidly enough or deeply enough, resulting in low blood oxygen levels and high blood carbon dioxide (CO_2) levels. Many people with this condition also frequently stop breathing altogether for short periods of time during sleep (obstructive sleep apnea), resulting in many partial awakenings during the night, which leads to continual sleepiness during the day (Olson AL, Zwillich C, 2005).

Obesity hypoventilation syndrome is defined as the combination of obesity (body mass index above 30 kg/m^2), hypoxia (falling oxygen levels in blood) during sleep, and hypercapnia (increased blood carbon dioxide levels) during the day, resulting from hypoventilation (excessively slow or shallow breathing)(Mokhlesi B et al., 2007; American Academy of Sleep Medicine, 1999).

1.3 Absorption of dietary lipids and pancreatic lipase inhibitors

Lipid metabolism normally maintains an elegant balance between synthesis and degradation. When the balance is lost, hyperlipidemia, such as hypertriglyceridemia and hypercholesterolemia, may develop. This can cause variety of serious diseases, such as arteriosclerosis, hypertension, obesity, diabetes, functional depression of some organs, etc (Bray *et al.,* 2002).

Pancreatic lipase (PL) (triacylglycerol hydrolase, EC 3.1.1.3) is a key enzyme for lipid breakdown required to absorb fatty acids (Thomson, 1993). Pancreatic lipase, one of the exocrine enzymes of the pancreatic juice, catalyzes the hydrolysis of emulsified esters of glycerol and long-chain fatty acids (Figure 3).

Short-chain fatty acids can be directly absorbed into the blood, while long-chain fatty acids and monoglycerides combine with bile salts to form water soluble micelles, see figure 4 (Carey, 1983). The micelles are absorbed into the mucosal cells of the intestine, and the fatty acids and monoglycerides are resynthesized into triglycerides. These triglycerides are formed into small particles known as chylomicrons, which consist of triglycerides, the sterol lipid cholesterol, and apoproteins. These chylomicrons are transported to the muscle and adipose tissue. Dietary triglyceride is usually stored in the adipose tissue.

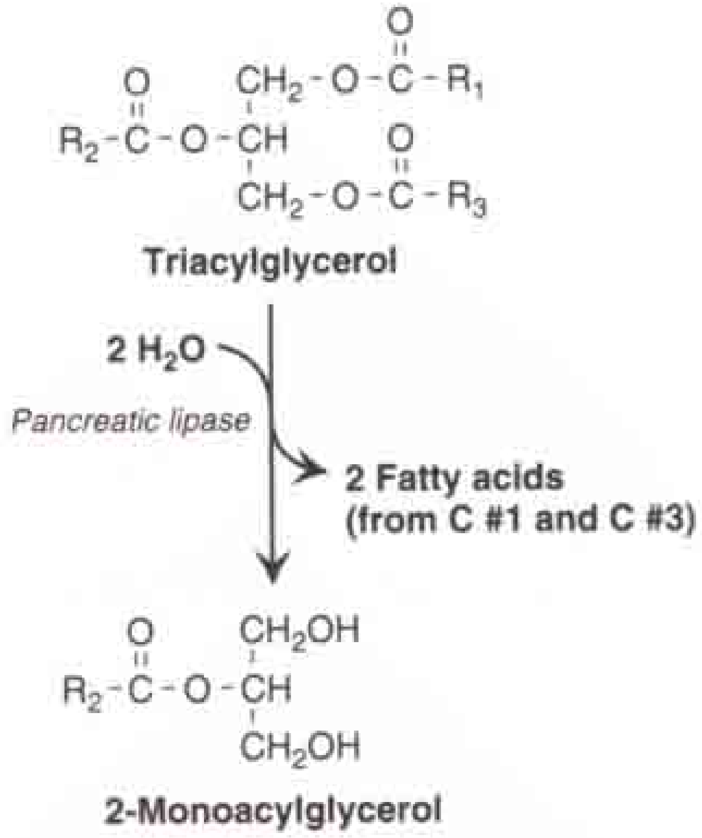


Figure 3: Triglycerides are hydrolyzed into 2 fatty acids and 2-monoacylglycerol by the enzyme pancreatic lipase. Inhibition of pancreatic lipase will interfere with triglycerides absorption.

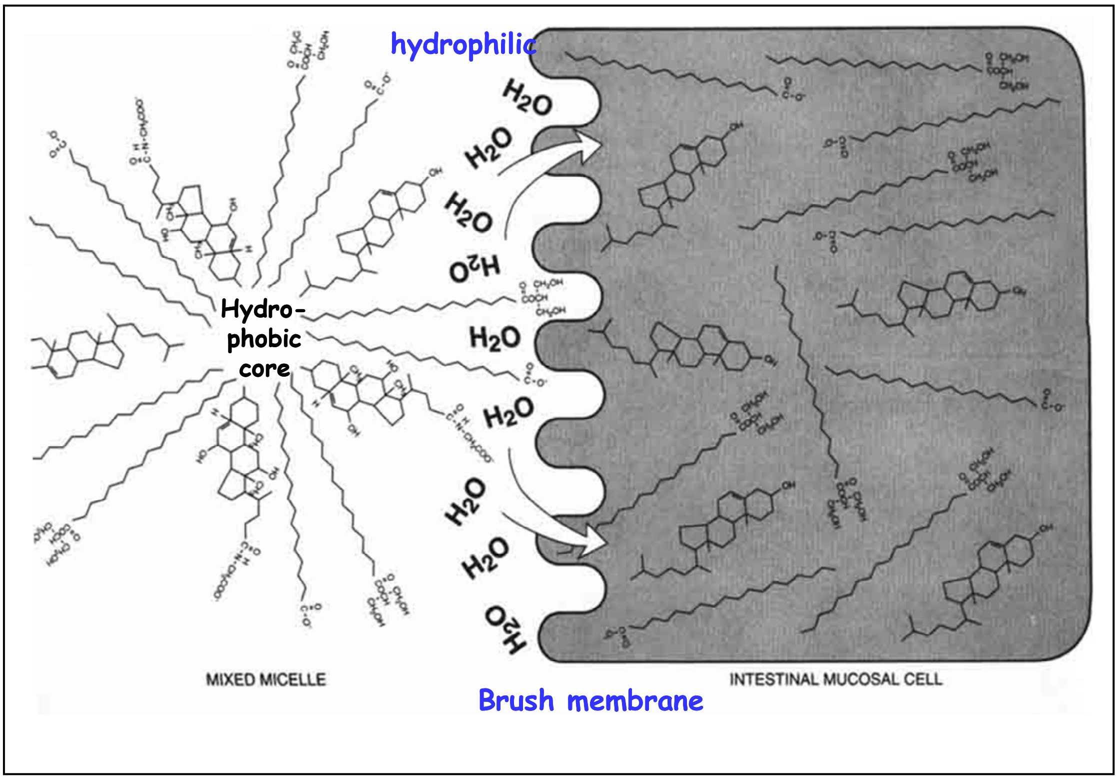


Figure 4: The primary products of dietary lipid degradation with the bile salts from mixed micelles. The components of mixed micelle will diffuse to the intestinal epithelial cells.

Inhibition of pancreatic lipase deprives the body of ingested calories because unhydrolyzed triacylglycerols are not absorbed from the intestine. Orlistat (Xenical®), a pancreatic lipase inhibitor, is a hydrogenated derivative of lipstatin from *Streptomyces toxytricini*, it binds covalently to the active site serine of pancreatic lipase (Hadvary, 1991). Administration of orlistat in patients has resulted in up to a 25% decrease of fat absorption resulting in a loss of body weight. Lipase inhibiting compounds other than orlistat have been developed, and polymers that bind fat to prevent its absorption have also been reported (Jandacek, 2004).

The hydrolysis of triacylglycerol by lipase occurs at an interface between water and oil; therefore, any substance decreasing the interface area of the lipid substrate may also act as an inhibitor of lipase. Natural products such as the proteins from soybean seed and herring sperm as well as low molecular weight substances such as condensed

tannin from *Cassia mimosoides*, flavonoid from *Citrus unshiu*, an ethyl acetateextract, caulerpenyne, from *Caulerpa taxifolia*, and catechins from oolong tea have been reported to inhibit lipase activity (Gurib-Fakim, 2006). Many of these inhibitors are amphoteric substances, which could influence the nature of the interface of the lipid substrate. However, the PL-inhibitory activities of natural herbal products have not been studied (Gurib-Fakim, 2006).

An alternative approach to reduce absorption of ingested fat has been the development of fat that cannot be hydrolyzed. Olestra (brand name Olean®), a sucrose molecule esterified with six to eight long-chain fatty acids, has the physical properties of dietary fats but is not hydrolyzed in the intestine (Wassan, 2005).

This approach minimizes gastrointestinal side effects so long as the unabsorbed fat contains appropriate high-melting fatty acids, although gastrointestinal side effects can persist (Jandacek, 1999).

The resistance to lipase also prevents hydrolysis catalyzed by bacterial lipases in the colon so that irritant fatty acids are not produced.

2. Importance of the Study

As mentioned previously, obesity is considered to be the most prevalent metabolic disease in the world. As a result, of civilization there have been important changes in lifestyle leading to a progressive decline in the necessity of physical activity. The consequence is a rising trend in its prevalence worldwide, not only in adults but also, and more concerning, in childhood. This increase in obesity prevalence has important consequences in terms of public health because of the concomitant increase in the multiple non communicable diseases associated with obesity. These include metabolic disturbances such as type 2 DM, dislipemia and hyperuricemia, as well as cardiovascular diseases, being hypertension, ischaemic heart disease and stroke, the more common. Nevertheless, obesity and particularly central type obesity can affect almost every organ and system of the body.

Respiratory disturbances, often misdiagnosed in obese people, can severely affect their health and quality of life. This is the case for obesity hypoventilation syndrome and the better known obstructive sleep apnea syndrome. We are confronting a real obesity epidemic and major efforts should be driven towards a better understanding about the causes of obesity so that we can find effective and safe tools to treat obese patients. Otherwise, in a few decades the world will be obese with a tremendous impact in terms of public health and economic costs.

Although a number of pharmacological approaches have been investigated in recent years, few therapeutically effective and safe products have been developed (Jandacek and Woods, 2004). The high incidence of obesity and the lack of safe pharmaceutical agents have promoted us to study and evaluate plant *Salvia triloba* extract that able to inhibit the pancreatic lipase. Identification of new, safe anti-obesity agent will be our

target because of its significance in improving the life style of humans, and because of its marketing value. In fact, Orlistat (Xenical®) which inhibits pancreatic lipase and used for obesity treatment is one of the best selling medications in the world.

3. Materials and methods

3.1 Solution and Materials

- Lipase from Porcine Pancreas, Type 2 (PPL,Sigma, USA).
- Tris base (Promega, USA).
- Para-nitrophenyl butyrate (PNPB, Sigma, USA).
- Dimethyl sulphoxide (DMSO,Tedia, USA).
- Acetonitrile (ACN, Tedia, USA).
- Diethyl ether (SDFCL, Mumbai).
- Hydrogen Chloride (HCl, SDFCL, Mumbai).
- Methanol (MeoH, Scharlau chemie S.A.,Barcelona, Spain).
- Ethanol (EtOH, EC).
- Sodium Chloride (NaCl, Gainland chemical, UK).
- Orlistat (assay 99.1%, United Pharmaceutical, Jordan).

3.2 Apparatus and Equipment

- Spectrophotometer Unicam UV2-100 UV/Vis, Unicam, England.
- Centrifuger, Sigma, Germany.
- Centrifuger, Centurion Scientific, UK.
- Electronic Balances, Shimadzu, Japan.
- Hotplate and Stirrer, Ikamag RCT, Germany.
- Lithium heparin tubes, Aptaca, Italy.

3.3 Methods

3.3.1 Plant Extraction:

Salvia triloba (Sage) were purchased from local supermarketin summer and identified by Doctor Khaled Tawaha (Department of Phytochemistry, Jordan University).

The leaves weredried for three days and then finely powdered. The powder wasextracted with absolute methanol in a Soxhletapparatus for 72 h.

After extraction, the solvent was filteredand then evaporated by Rotavapor.The obtained sage alcoholic extract putted at the pinch at home temperature for 24 hr to obtain full evaporation and then stored in refrigerator until usage.

3.3.2 Measurement of Pancreatic Lipase Activity:

The pancreatic lipase activity can be measured by colorimetric assay that measures the release of p-nitrophenol using p-nitrophenyl butyrate (PNPB)as a substratewith some modification (Slee, D. H, 2003).

Briefly, the reaction mixture containing test plant extract solution in DMSO (20µL), the buffer reagent (875µL) and the enzyme solution (100µL), was mixed, then the substrate solution (5µL) wasadded to start the reaction and mixed for half minute and measured at 410 nm. The IC50 value wasdetermined graphically by a plot of percent inhibitionsversus log concentrations of the test plant sample.

Serial dilution was done for plant extract solution so that the concentrations were 6.25, 12.5, 25, 50, and 200 µg/mL .

Blank solution contains 20 μL of the respective plant extract concentration in sample test tube and 980 μL buffer solution.

Un-inhibitory control was also done and it contain DMSO (20μL), the buffer reagent (875μL), the enzyme solution (100μL), and the substrate solution (5μL).All measurements were done when the PNPB substrate were added at 410 nm using double beam spectrophotometer.

3.3.3Subjects

Male Albino rats initially weighing 375-425 g (average 400g) were used. The rats were housed in groups of 5 per cage with free access to tap water. At first, male rats were fasted for 24 h, and then 1 ml mixture (contains 20% ethanol and 80% water plus the extract at 750mg/kg or orlistat 5mg/kg) was given to the rats by oral-intubation.

After half an hour, fat (50g) were putted for every cage for eating up to 2 hours. Then, the residual fat were collected and weighed for determination of eaten fat weight.

Blood samples were collected from the tail vein under ether anesthesia at 2 h after adding fat and centrifuged at rpm4000 for 5 min toobtain the plasma.Plasmatriglyceride (TG) levels were determined using a commercial kit (Joaquim Costa, Barcelona, Spain).

Rats are divided into four groups(five rats in each group):

1. Rats on fat : (Negative control Group)
2. Rats on fat and orlistat (5mg/kg) : (Positive control Group)
3. Rats on fat and plant extract(750mg/kg) : (Test Group)
4. Rats fasting

3.3.4 Triglyceride Kit Principle

The method (Buccolo G and david H, 1982) is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL).

The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerolkinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP).

G-3-P is oxidized by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide.

A red chromogen is produced by the peroxidase (POD) catalyzed coupling of4-aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H_2O_2), proportional to the concentration of triglyceride in the sample.

$$\text{Triglycerides} + 3\,H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + 3\text{FFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{G-3-P} + \text{ADP}$$

$$\text{G-3-P} + O_2 \xrightarrow{\text{GPO}} \text{DHAP} + H_2O_2$$

$$\text{4-AA} + 4\ \text{Phenol} \xrightarrow{\text{H2O2, POD}} \text{Quinoneimine} + H_2O$$

4. Results and Discussion

4.1 *In vitro* results

The effect of extract on lipase activity were examined using control and different concentration from the plant extract.

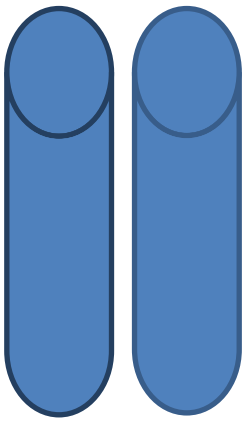


Figure 5: control and blank composition

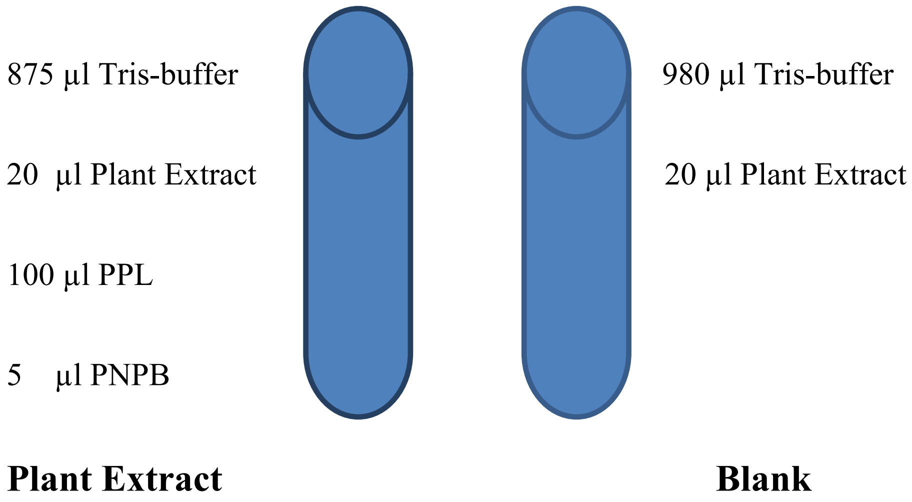


Figure 6: Plant Extract and blank composition

- Solutions of the Control test were added in this order:

1. Tris-buffer
2. DMSO
3. PPL
4. PNPB

- Solutions of the Plant Extract test tube were added in this order:

1. Tris-buffer
2. Plant Extract
3. PPL
4. PNPB

*When PNPB were added, the measurement starts

4.1.1 Calculation of IC50

Equation and regression for all concentrations were:

- Control: $y=0.0749x+0.0124R^2 = 0.9996$
- Plant Extract (200 µg/ml): $y=0.0274x+0.2523R^2 = 0.9954$
- Plant Extract (50 µg/ml): $y = 0.0491x + 0.3397R^2 = 0.9934$
- Plant Extract (25 µg/ml): $y = 0.0514x + 0.4008R^2 = 0.995$
- Plant Extract (12.5 µg/ml): $y = 0.058x + 0.207R^2 = 0.9929$
- Plant Extract (6.25 µg/ml): $y = 0.0597x + 0.305R^2 = 0.9926$

Table 4 and Figure 7 summarize *in vitro* results.

Table 4: Percent of Inhibitory Concentration

Concentration of the plant Extract µg/mL	Rate of reaction for Extract(µg/ml.min)	% of Inhibitory Activity
6.25	0.0597	20.29372497
12.5	0.058	22.56341789
25	0.0514	31.37516689
50	0.0491	34.4459279
200	0.0274	63.41789052
Control	0.0749	0.0

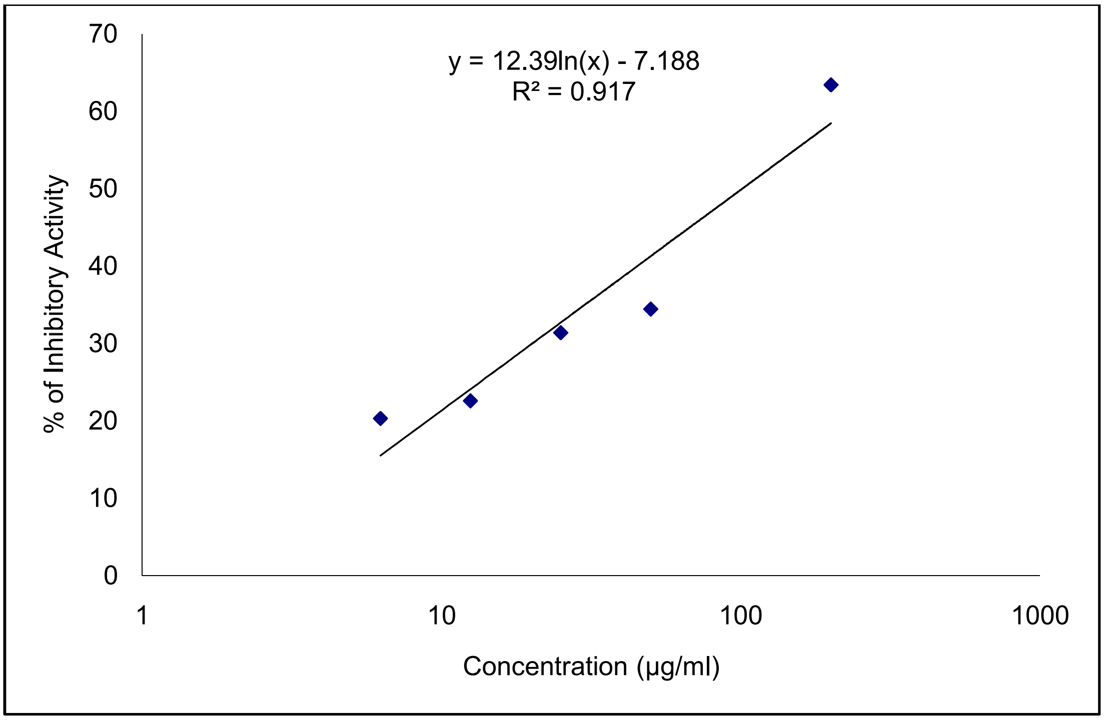


Figure 7: Percent of Inhibitory Activity (Y axis) and Plant Extract Concentration in µg/ml (X axis) in a Logarithmic scale.

As we can see that as the extract concentration decreases, the rate of the reaction increases, and all rate of the reactions are less than the control that does not have any anti-lipase activity.

IC50 is a quantitative measure indicates how much of the plant extract is needed to inhibit Pancreatic Lipase by half.

Percent of Inhibitory Activity was calculated according to the following formula% of inhibition = 100 – (Rate of Reaction in the presence of theextract\ Rate of Reaction of the control * 100%).

From figure 7, we can calculate the IC50 as following:

y = 12.393ln(x) - 7.1887, so IC50: 100.9 µg/ml.

4.2 *In vivo* Results

As previously mentioned, there were four cages, each cage contains 5albimo rats with average weight of 400g.

1. The first cage contains five rats was given the mixture with Orlistat 5mg/kg (Positive Control).
2. The second cage contains five rats was given the mixture with Plant Extract 750 mg/kg (Test).
3. The third cage contains five rats was given just the mixture without Orlistat or Extract (Negative Control).
4. The fourth cage contains five rats was not given any thing (Fasting)

The amount of eaten fat was:

- The first cage which was given the mixture with Orlistat 5mg/kg (Positive Control) was 14g.
- The second cage which was given the mixture with Plant Extract 750 mg/kg (Test) was 16g.
- The third cage which was given just the mixture without Orlistat or Extract (Negative Control) was 17.

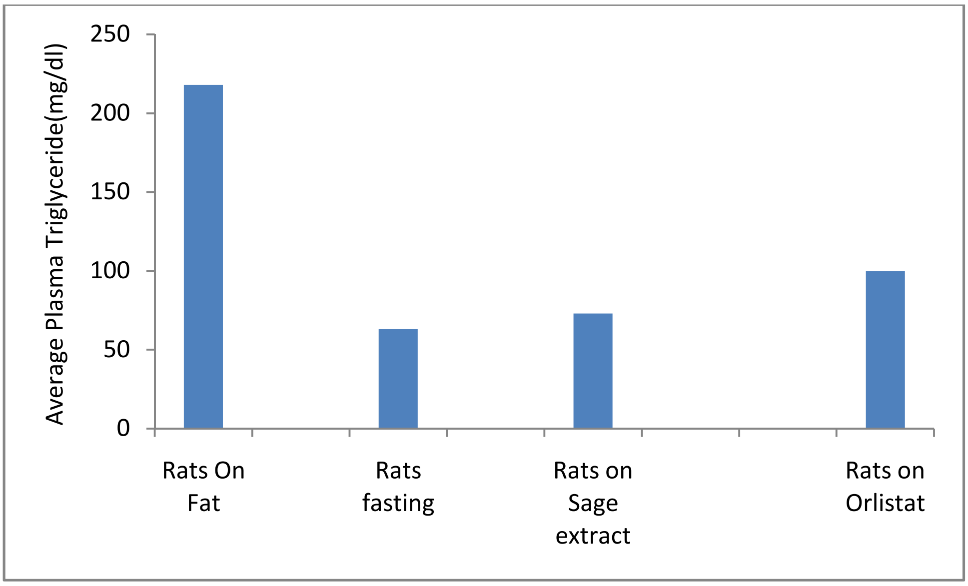


Figure 8: Average Plasma Triglyceride vs cage group.

From *in vivo* results, there was significant decrease in the plasma triglycerides level for rats take Orlistat or Extract compared to rats take only fat (P-value 0.0381 and 0.0092 respectively).Indeed, no significant difference between rats take Orlistat or Extract compared to fasting rats (P-value 0.2224 and 0.5928 respectively). Also, there was significant difference between fasting rats and rats take only fat (P-value 0.0042). In addition to that, no significant difference between rats take Orlistat and rat take Extract (P-value 0.464).

5. Conclusions and Recommendations

5.1 Conclusion

In conclusion, there was pancreatic lipase inhibition activity from the leaves of *S.triloba* which was confirmed *in vivo* by the suppression of plasma TG elevation in fat-fed rats. Also, in vitro analysis shows that*S.triloba* extract has anti-pancreatic lipase activity with IC50: 100.9 µg/ml.

Therefore, *Salvia triloba* could be effective for control of obese patients due to over intake of high fat diet.

5.2 Recommendations

Further studies on *S.triloba*extract are needed to identify the exact constituents of this widely used plant in our country.

References

Abou-Jawdah Y, Sobh H, Salameh A (2002) Antimycotic activities of selected plant flora, growing wild in Lebanon, against phytopathogenic fungi. **J Agric Food Chem**50:3208–3213.

Adam K, Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M (1998). Antifungal activities of *Origanum vulgare subsp. hirtum, Mentha spicata, Lavandula angustifolia, and Salvia fruticosa* essential oils against human pathogenic fungi**. J Agric Food Chem** 46:1739–1745.

Adami HO & Trichopoulos D. Obesity and mortality from cancer. **N Engl J Med** 2003; 348: 1623–1624.

Amad KH, Brennan JC, Alexander JK. The cardiac pathology of chronic exogenous obesity. **Circulation** 1965; 32(5):740-5.

American Academy of Sleep Medicine (2001). *The International Classification of Sleep Disorders*. Westchester, Illinois: American Academy of Sleep Medicine, pp. 52–8. Retrieved 2010-09-11.
http://en.wikipedia.org/wiki/Obstructive_Sleep_Apnea

American Academy of Sleep Medicine 1999. "Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. *Sleep* 22 (5): 667–89.**http://en.wikipedia.org/wiki/Obesity_hypoventilation_syndrome**

Ahmad S, Kapadia Z, Badar Y. **Fitoterapia** 1994; 65:271.

Ahmadi, L., & Mirza, M. (1999). Essential oil of Salvia multicaulis Vahl from Iran. **Journal of Essential Oil Research**, 11, 289–290.

Ali-Shtayeh MS, Abu Ghdeib SI (1999) Antifungal activity of plant extracts against dermatophytes. **Mycoses** 42:665–672.

Al-Junaidy MJ (1993) **Medicinal, Aromatic, and Industrial Wild Plants of Jordan and their Benefits**. Al-Assail Publisher, Amman, Jordan.

American Diabetes Association. Diagnosis and classification of diabetes mellitus. **Diabetes Care** 2004; 27(supplement 1): S5–10.

Ana Paula Longaray Delamare , Ivete T. Moschen-Pistorello, Liane Artico, Luciana Atti-Serafini, Sergio Echeverrigaray, 2007. **Food Chemistry** 100 (2007) 603–608.

Anderson JJ & Felson DT. Factors associated with osteoarthritis of the knee in the first National Health and Nutrition Examination Survey (NHANES I). Evidence for an association with overweight, race, and physical demands of work. **Am J Epidemiol** 1988; 128: 179–189.

Appleton SL, Adams RJ, Wilson DH, Taylor AW, Ruffin RE. North West Adelaide Cohort Health Study Team. Central obesity is associated with nonatopic but not atopic asthma in a representative population sample. **J Allergy Clin Immunol** 2006;118:1284—91.

Arita Y, Kihara S, Ouchi N et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. **Biochem Biophys Res Commun** 1999; 257: 79-83.

Arnold, N., Bellomaria, B., 1993. Observations morphoanatomique et histochimique de *Salvia willeana* (Holmboe) Hedge et *Salvia fruticosa* Mill. de Chypre. **Flora Mediterranea** 3, 283–297.

Arnold, W.N., 1988. Vincent van Gogh and the thujone connection. **Journal of the American Medical Association** 260 (20), 3042–3044.

A.Bayrak and A. Akgul, **Phytochemistry**, 26, 846 (1987).

A. Elbetieha,M.H. Al-Hamood, A. Alkofahi, H. Bataineh 1998. **Journal of Ethnopharmacology** 61 (1998) 67–74.

Bahar Ulubas, Ramazan Gen , Munir Tumkaya ,Esen Akbayb, Mukadder Calıkoglua. **Obesity Research & Clinical Practice** (2011) 5, e79-e83.

Baik I, Ascherio A, Rimm EB et al. Adiposity and mortality in men. **Am J Epidemiol** 2000; 152: 264–271.

Baser, K., Honda G., Miki, W., 1986. **Herb drugs and herbalists in Turkey**. Institute for the study of languages and cultures of Asia and Africa, Tokyo.

Bellomaria, B., Arnold, N., Valentini, G., 1992. Contribution to the study of the essential oils from three species of *Salvia* growing wild in the Eastern Mediterranean region. **Journal of Essential Oil Research** 4, 607–614.

Bhargava SK, Sachdev HS, Fall CH et al. Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. **N Engl J Med** 2004; 350: 865–875.

Blumenthal, M., Goldberg, A., Brinckmann, J., 2000. Herbal Medicine: Expanded Commission E Monographs. **Integrative Medicine Communications**, USA.

Booth ML, Wake M, Armstrong T, Chey T, Hesketh K, Mathur S. The epidemiology of overweight and obesity among Australian children and adolescents, 1995-97. **Aust N Z J Public Health** 2001; 25:162-9.

Boyko EJ, FujimotoWY, Leonetti DL & Newell-Morris L. Visceral adiposity and risk of type 2 diabetes: a prospective study among Japanese Americans. **Diabetes Care** 2000; 23: 465-471.

Bray, G. A., and Tartaglia, LA. **Nature** 2002, 404: 672-677.

Brieskorn, C.H., Klinger, H., Polonius, W., 1961. U ¨ ber das gemeinsame Vorkommen von Triterpenen und Sterinen im Blatt von *Salvia triloba* und Pirus malus. **Arch. Pharm.** 7 294/66.

Brieskorn, C.H., 1991. Salbei seine Inhaltsstoffe und sein therapeutischer Wert. Z. **Phytother**. 12, 61–69.

Buccolo G and david, H. Clin. Chem. 1973, Fossati, R. and Prencipe, L. **Clin. Chem.** 1982.

Budavari, S., 1989. **The Merck Index: An Encyclopedia of Chemicals, Drugs and Biological**, 11th ed. Merck & Company, Rahway, NJ, USA.

Burnham Institute for Medical Research (2007, November 2). Rosemary Chicken Protects Your Brain From Free Radicals. ScienceDaily.**http://en.wikipedia.org/wiki/Rosemary**

Cabo, Y., Crespo, M.E., Jimenez, J., Navarro, C., Perani, E., Zarzuelo, H., 1987. Sur l'huile essentille de *Salvia lavandulifolia vahl subsp.* Oxydon. **Plantes Medicinales et Phytotherapie** 21 (2), 116–121.

Calle EE, Rodriguez C,Walker-Thurmond K & Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. **N Engl J Med** 2003; 348: 1625–1638.

Calle EE, ThunMJ, Petrelli JMet al. Body-mass index and mortality in a prospective cohort of U.S. adults**. N Engl J Med** 1999; 341: 1097-1105.

Cardil V, Russo A, Formisano C, Rigano D, Senatore F, Apostolides Arnold N, et al. Essential oils of *Salvia bracteata* and *Salvia rubifolia* from Lebanon: chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. **J Ethnopharmacol** 2009; 126:265-72.

Carlson SH, Shelton J, White CR & Wyss M. Elevated sympathetic activity contributes to hypertension and salt sensitivity in diabetic obese Zucker rats. **Hypertension** 2000; 35: 403–408.

Carey M. C., Small D. M., Bliss C. M., Annu. Rev. **Physiol.** 1983, 45: 651- 677.

Clebsch, Betsy; Carol D. Barner (2003). The New Book of Salvias. Timber Press. pp. 125–127.**http://en.wikipedia.org/wiki/Salvia_triloba**

Coggon D, Reading I, Croft P et al. Knee osteoarthritis and obesity. **Int J Obes Relat Metab Disord** 2001; 25:622–627.

Codd, L.E.W., 1985. Lamiaceae: **Flora of Southern Africa**. 28 Botanical Research Institute, Pretoria.

Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL (February 1996). "Serumimmunoreactive-leptin concentrations in normal-weight and obese humans". **N. Engl. J. Med.** 334 (5): 292–5.

Creager MA, Cooke JP, Mendelsohn ME, Gallagher SJ, Coleman SM, Loscalzo J, et al. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. **J Clin Invest** 1990; 86:228-34.

Csendes A, Burdiles P, Smok G. et al. Histologic findings of gallbladder mucosa in 87 patients with morbid obesity without gallstones compared to 87 control subjects. **J Gastrointest Surg** 2003; 7: 547–551.

Daferera DJ, Ziogas BN, Polissiou MG (2000) GC–MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. **J Agric Food Chem** 48:2576–2581.

Dal Maso L, Augustin LS, Karalis A et al. Circulating adiponectin and endometrial cancer risk. **J Clin Endocr Metab** 2004; 89: 1160–1163.

Dapkevicius, A., Venskutonis, R., Beek, T., Linssen, J., 1998. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. **Journal of the Science of Food Agriculture** 77, 140–146.

Darias V, Bravo L, Rabanal R, Sanchez-Mateo CC, Martin-Herrera DA. **Planta Med** 1990; 56:70.

De Jong PE, Verhave JC, Pinto-Sietsma SJ & Hillege HL. Prevend study group. Obesity and target organ damage: the kidney. **Int J Obes Relat Metab Disord** 2002; 26(supplement 4): S21–24.

Denke MA, Sempos CT & Grundy SM. Excess body weight. An underrecognized contributor to high blood cholesterol levels in white American men. **Arch Intern Med** 1993; 153: 1093–1103.

Despres JP, Lemieux I & Prud'homme D. Treatment of obesity: need to focus on high risk abdominally obese patients. **BMJ** 2001; 322: 716–720.

Dowling HJ & Pi-Sunyer FX. Race-dependent health risks of upper body obesity. **Diabetes** 1993; 42: 537–543.

Ebbeling CB, Pawlak DB, Ludwig DS. Childhood obesity: public-health crisis, common sense cure. **Lancet** 2002;360: 473-82.

Eckel RH. Obesity and Heart disease. A statement for healthcare professionals from the Nutrition Committee, Amearican Heart Association. **Circulation** 1997; 96: 3248–3250.

Eleonora Dal Grandea, Tiffany Gilla, Lydia Wyatta, Catherine R. Chittleborougha, Patrick J. Phillipsb, Anne W. Taylora, 2009. **Obesity Research & Clinical Practice** (2009) 3, 159—168.

Elisabetsky, E., Marschner, J., Souza, D.O., 1995. Effect of linalool on glutamatergic system in the rat cerebral cortex. **Neurochemical Research** 20 (4), 461–465.

El-Atat F, Aneja A, Mcfarlane S & Sowers J. Obesity and hypertension. **Endocrinol Metab Clin N Am** 2003; 32: 823–854.

Engeli S & Sharma AM. The renin-angiotensin system and natriuretic peptides in obesity-associated hypertension. **J Mol Med** 2001; 79: 21–29.

European Pharmacopoeia, 1971. vol. II, European Treaty Series No. 50, Maisonneuve, S.A. Publishers, France.

Evert-Ting, W.T., Deibel, K.E., 1992. Sensitivity of Listeria monocytogenes to spices at two temperatures. **Journal of Food Safety** 12 (2), 129–137.

Exarchou V, Nenadis N, Tsimidou M, Gerothanassis IP, Troganis A, Boskou D (2002). Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage, and summer savory. **J Agric Food Chem** 50:5294–5299.

E. Sezik and E. Yesilada, **Essential Oils**, In honour of Prof. Dr. K. H. C. Baser on his 50th birthday (Eds. N. Krimer, A. Mat), 1999, 98.

Farooqi IS, Keogh JM, Kamath S, Jones S, GibsonWT, Trussell R, et al. Partial leptin deficiency and human adiposity. **Nature** 2001; 414:34-5.

Felson DT, Anderson JJ, Naimark A et al. Obesity and knee osteoarthritis. The Framingham Study. **Ann Intern Med** 1988; 109: 18–24.

Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG. Osteoarthritis: New insights. Part 1: The disease and its risk factors. **Ann Intern Med** 2000; 133:635—46.

Feskens EJM, Toumilehto J, Stengard JH et al. Hypertension and overweight associated with hyperinsulinaemia and glucose tolerance: a longitudinal study of the Finnish and Dutch cohorts of the Seven Countries Study. **Diabetologia** 1995; 38: 839–847.

Filozof C, Gonzalez C, Sereday M, et al. Obesity prevalence and trends in Latin-American countries. **Obesity Rev** 2001;2:99—106.

Fragaki, E., 1969. **Contribution in the vernacular name of native, naturalised, pharmaceutical, dye, ornamental and edible plants of Crete**. Athens (in Greek).

Friedrich MJ. Researchers explore obesity-cancer link. **JAMA** 2003; 290: 2790–2791.

Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. **J Clin Endocrinol Metab** 1998; 83:847-50.

Gali-Muhtasib,H.,Hilan, C., Khater, C., 2000. Traditional uses of Salvia libanotica (East Mediterranean sage) and the effects of its essential oils. **Journal of Ethnopharmacology** 71, 513–520.

Gami AS, Caples SM & Somers VK. Obesity and obstructive sleep apnea. **Endocrinol Metab Clin N Am** 2003; 32: 869–894.

Gami AS, Hodge DO, Herges RM, Olson EJ, Nykodym J, Kara T, et al. Obstructive sleep apnea, obesity, and the risk of incident atrial fibrillation. **J Am Coll Cardiol** 2007; 49(5): 565-71.

Geller, R.J., Spyker, D.A., Garretson, L.K., Rogol, A.D., 1984. Camphor toxicity: development of a triage strategy. **Veterinary and Human Toxicology** 26 (Suppl. 2), 8–10.

Gennaro, A.R., 1990. **Remington's Pharmaceutical Sciences**, 18th ed. Mack, Easton, PA.

Geunther, E., 1949. **The Essential Oils**, vol. I. Van Nostrand, New York.

Goldstein BJ, Ahmad F, Ding W et al. Regulation of the insulin signaling pathway by cellular protein tyrosine phosphatases. **Mol Cell Biochem** 1998; 182: 91-99.

Greuter, W., Burdet, H. M., Long, G., 1986. **Med-Checklist**, vol. 3. Editions de Conservatoire de Jardin Botaniques de la Ville de Geneve.

Griffin ME, Marcucci MJ, Cline GW et al. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. **Diabetes** 1999; 48:1270-1274.

Grolier Multimedia Encyclopedia, 1992. Grolier Interactive Inc.

Grundy SM, Hansen B, Smith Jr SC, Cleeman JI, Kahn RA, American Heart Association; National Heart, Lung, and Blood Institute; American Diabetes Association. Clinical management of metabolic syndrome: report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management. **Circulation** 2004; 109:551-6.

Gurib-Fakim . A., Mol. Aspec. **Med.** 2006, 27: 1–93.

Hadvary, P.; Sidler, W.; Meister, W.; Vetter, W.; Wolfer, H., **J. Biol. Chem.** 1991, 266: 2021-2025.

Hainault I, Nebout G, Turban S, Ardouin B, Ferre P, Quignard-Boulange A. Adipose tissue-specific increase in angiotensinogen expression and secretion in the obese (fa/fa) zucker rat. **Am J Physiol Endocrinol Metab** 2002; 282:E59-66.

Harborne, J. B. 1997. Plant secondary metabolism, pp. 132–155, in M. J. Crawley (ed.). **Plant Ecology**. Blackwell Science, Oxford.

Hasan MG, Al-Hazimi X, Maiana GA. **J Chem Soc Pak** 1986; 8:549.

Havakis I., 1978. **Plants and Herbs of Crete**. Athens (in Greek).

Hedge, I.C., 1974. **A Revision of Salvia in Africa IncludingMadagascar and the Canary Islands**. Royal Botanic Garden, Edinburgh.

Hefnawy, Y., Moustafa, S., Marth, E., 1993. Sensitivity of Listeria monocytogenes to selected spices. **Journal of Food Protection** 56 (10), 876–878.

Hilan, C., Khazzaka, K., Sfeir, R., 1997. Antimicrobial effect of essential oil of Salvia libanotica (Sage). **British Journal of Phytotherapy** 4 (4), 1–3.

Hoffstedt J, Arvidsson E, Sjolin E et al. Adipose tissue adiponectin production and adiponectin serum concentration in human obesity and insulin resistance. **J Clin Endocrinol Metab** 2004; 89: 1391-1396.

Hooser, S.B., 1990. Toxicology of selected pesticides, drugs, and chemicals. D-limonene, linalool, and crude citrus oil extracts. **Veterinary Clinics of North America – Small Animal Practice** 20, 383–385.

Hotamisligil GS. The role of TNF-α and TNF receptors in obesity and insulin resistance. **J Intern Med** 1999; 245: 621-625.

Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity linked insulin resistance. **Science** 1993; 259:87-91.

Huang Z, WilletWC, Manson JE et al. Body weight, weight change, and risk for hypertension in women. **Ann Intern Med** 1998; 128: 81–88.

Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. **Circulation** 1983; 67:968-77.

Hu FB, Manson JE, Stampfer MJ et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. **N Engl J Med** 2001; 345: 790-797.

Jandacek RJ, Woods SC. **Drug Discov.** Today 2004, 15: 874-880.

Jandacek R.J., et al., **Lipids** 1999, 34: 771– 783.

Jaquet D& Czernichow P. Born small for gestational age: increased risk of type 2 diabetes, hypertension and hyperlipidaemia in adulthood. **Horm Res** 2003; 59(supplement 1): 131–137.

Jelnar Z. Al–Kalaldeh, Rana Abu–Dahab, Fatma U. Afifi, 2010. **Nutrition Research** 30 (2010) 271–278.

Johnson J.J. "Carnosol: A promising anti–cancer and anti–inflammatory agent" **Cancer Letters** 2011 305:1 (1–7).

Junghyun Kim, Dae Sik Jang, Hyojun Kim, and Jin Sook Kim, 2009. **Diabetic Complications Research Center**, Division of Traditional Korean Medicine (TKM) Integrated Research, Korea Institute of Oriental Medicine, Daejeon 305–811, Korea.

Kahn BB & Flyer JS. Obesity and insulin resistance. **J Clin Invest** 2000; 106: 473–481.

Kaileh M, Berghe WV, Boone E, Essawi T, Haegeman G (2007). Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity. **J Ethnopharmacol** 113:510–516.

Kambham N, Markowitz GS, Valeri AM et al. Obesity-related glomerulopathy: an emerging epidemic. **Kidney Int** 2001; 59: 1498–1509.

Kamatou G, Van Zyl R, Davids H, Van Heerden F, Lourens A, Viljoen A. Antimalarial and anticancer activities of selected South African *Salvia* species and isolated compounds from *S.radula*. **S Afr J Bot** 2008;74:238–43.

Karim, F., Quraan, S., 1986. **Medicinal Plants of Jordan**. Centre for Jordanian Studies, Irbid.

Kbayssi, H., 1993. **Encyclopedia of Weeds and Medicinal Plants**. Scientific Books, Beirut, Lebanon, p. 271.

Kenchaiah S, Evans JC, Levy D, Wilson PW, Benjamin EJ,Larson MG, et al. Obesity and the risk of heart failure. **N Engl J Med** 2002;347(5):305—13.

Kintzios, S., Nikolaou, A., Skoula,M., 1999. Somatic embryogenesis and in vitro rosmarinic acid accumulation in Salvia officinalis and S. fruticosa leaf callus cultures. **Plant Cell Rep**. 18, 462} 466.

Kiyofumi Ninomiya, Hisashi Matsuda, Hiroshi Shimoda, Norihisa Nishida, aoki Kasajima, Tomoe Yoshino, Toshio Morikawaa and Masayuki Yoshikawa. **Bioorganic & Medicinal Chemistry Letters** 14 (2004) 1943–1946.

Knudson JD, Dincer UD, Zhang C, Swafford Jr AN, Koshida R, Picchi A, et al. Leptin receptors are expressed in coronary arteries, and hyperleptinemia causes significant coronary endothelial dysfunction. **Am J Physiol Heart Circ Physiol** 2005; 289(1):H48–56.

Laitinen J, Sovio U. Nuorten lihavuuden kehityssuunnat.**Suomen Lääkärilehti** 2005; 60:4231–5.

Lakka HM, Lakka TA, Tuomilehto J & Salonen JT. Abdominal obesity is associated with increased risk of acute coronary events in men. **Eur Heart J** 2002; 23: 706–713.

Larsson UE & Mattsson E. Perceived disability and observed functional limitations in obese women. **Int J Obes Relat Metab Disord** 2001; 25: 1705–1712.

Laude, E.A., Morice, A.M., Grattan, T.J., 1994. The antitussive effects of menthol, camphor and cineole in conscious guinea pigs. **Pulmonary Pharmacology** 7 (3), 179–184.

Laura R. Aballaya, Alberto R. Osellab, Alejandra Celi, María del Pilar Díaza. **Obesity Research & Clinical Practice** (2009) 3, 75—83.

Lean MEJ, Han TS & Morrison CE. Waist circumference as a measure for indicating need for weight management. **BMJ** 1995; 311: 158–161.

Lemieux I, Pascot A, Couillard C et al. Hypertriglyceridemic waist. A marker of the atherogenic metabolic triad (hyperinsulinemia, hyperapolipoprotein B, small, dense LDL) in men? **Circulation** 2000; 102: 179–184.

Leushner, J., 1997. **Reproductive toxicity studies of D–camphor in rats and rabbits**. Arzneimittelforschung 47 (2), 124–128.

Liu, J., 1995. Pharmacology of oleanolic acid and ursolic acid. **J. Ethnopharmacol**. 49, 57–68.

Liu X, Zu Y, Fu Y, Tao L, Gu C,WangW, et al. Antimicrobial activity and cytotoxicity towards cancer cells of *Melaleuca alternifolia* (tea tree) oil. **Eur Food Res Technol** 2009;229:247–53.

Longaray Delamare AP, Moschen–Pistorello IT, Artico L, Atti–Serafini L, Echeverrigaray S (2007) Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. **Food Chem** 100:603–608.

Lorentzen MS, Vestbo J, Lange P. **Are obesity and overweight risk factors of asthma?** Ugeskr Laeger 2001;163:4999—5003.

Maddox DA, Alavi FK, Santella RN & Zawada Jr. ET. Prevention of obesity-linked renal disease: age-dependent effects of dietary food restriction. **Kidney Int** 2002; 62: 208–219.

Manson JE, Willet WC, Stampfer MJ et al. Body weight and mortality among women. **NEJM** 1995; 333: 677–685.

Manson JE, Colditz GA, Stampfer MJ, Willett WC, Rosner B, Monson RR, et al. A prospective study of obesity and risk of coronary heart disease in women. **N Engl J Med** 1990; 322:882-9.

Mantzoros C, Petridou E, Dessypris N et al. Adiponectin and breast cancer risk. **J Clin Endocr Metab** 2004; 89: 1102–1107.

March L, Bagga H. Epidemiology of osteoarthritis in Australia. **Med J Aust** 2004; 180:S6—10.

Marx J., **Science** 2003, 299:846-849.

Matsingou TC, Petrakis N, Kapsokefalou M, Salifoglou A (2003). Antioxidant activity of organic extracts from aqueous infusions of sage. **J Agric Food Chem** 51:6696–6701.

Matsushita Y, Yoshiike N, Kaneda F et al. Trends in childhood obesity in Japan over the last 25 years from the National Nutrition Survey. **Obes Res** 2004; 12: 205–214.

Matsuura F, Yamashita S, Nakamura T et al. Effect of visceral fat accumulation on uric acid metabolism in male obese subjects: visceral fat obesity is linked more closely to overproduction of uric acid than subcutaneous fat obesity. **Metabolism** 1998; 47: 929–933.

Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. **Arterioscler Thromb Vasc Biol** 2004; 24:29-33.

Meikle, R.D., 1985. **Flora of cyprus**. The Bentham-Moxon Trust 2, pp.1287-1299.

Melpomeni Skoula, Jamel E. Abbes, Christopher B. Johnson 2000. **Department of Natural Products, Mediterranean Agronomic Institute of Chania**, Alsylion Agrokepiou, Chania.

Millet, Y., Jouglard, J., Steinmetz, M.D., Tognetti, P., Joanny, P., Arditti, J., 1981. Toxicity of some essential plant oils. Clinical and experimental study. **Clinical Toxicology** 18 (12), 1485–1498.

Millet, Y., Tognetti, P., Lavaire-Perlovisi, M., Steinmetz, M.D., Arditti, J., Jouglard, J., 1979. Experimental study of the toxic convulsant properties of commercial preparations of essences of sage and hyssop. **Revue d'Electroen-cephalographie et de Neurophysiologie Clinique** 9 (1), 12– 18.

Miyashita Y, Shirai K, Itoh Y et al. Low lipoprotein lipase mass in preheparin serum of type 2 diabetes mellitus patients and its recovery with insulin therapy. **Diabetes Res Clin Pract** 2002; 56: 181–187.

Mokdad AH, Ford ES, Bowman BA et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. **JAMA** 2003; 289: 76–79.

Mokhlesi B, Kryger MH, Grunstein RR (February 2008). "Assessment and management of patients with obesity hypoventilation syndrome". Proc Am Thorac Soc 5 (2): 218–25.**http://en.wikipedia.org/wiki/Obesity_hypoventilation_syndrome**

Mombouli J-V, Vanhoutte PM. Endothelial dysfunction: from physiology to therapy. **J Mol Cell Cardiol** 1999;31:61—74.

More LL, Bradlee ML, Singer MR et al. BMI and waist circumference as predictors of lifetime colon cancer risk in Framingham Study adults. **Int J Obes Relat Metab Disord** 2004; 28: 559–567.

Morton, J.F., 1976. **Herbs and Spices**. Golden Press, New York.

Mouterde, P., 1970. **Nouvelle flore du Liban et de la Syrie**. Dar El Machrek, Beyrouth.

Natasha J. Howard, Anne W. Taylor, Tiffany K. Gill, Catherine R. Chittleborough. **Obesity Research & Clinical Practice** (2008) 2, 51-59.

Neel JV. Diabetes mellitus: a 'thrifty' genotype rendered detrimental by 'progress'? **Am J Hum Genet** 1962;14: 353–362.

Nehme, M., 1978. Wild Flowers of Lebanon. **National Council for Scientific Research**, Beirut, Lebanon 208 pp.

Nelson LR, Bulun SE (September 2001). "Estrogen production and action". *J. Am.* **Acad. Dermatol**. 45 (3 Suppl): S116–24.

Olshansky SJ, Passaro DJ, Hershow RC, et al. **N. Engl. J. Med.** 2005, 352: 1135-1137.

Olson AL, Zwillich C (2005). "The obesity hypoventilation syndrome".*Am. J. Med.* 118 (9): 948–56.**http://en.wikipedia.org/wiki/Obesity_hypoventilation_syndrome**

Ozcan M (2003). Antioxidant activities of rosemary, sage, and sumac extracts and their combinations on stability of natural peanut oil.**J Med Food** 6:267–270.

Paeratakul S, Lovejoy JC, Ryan DH & Bray GA. The relation of gender, race and socioeconomic status to obesity and obesity comorbidities in a sample of US adults. **Int J Obes Relat Metab Disord** 2002; 26: 1205–1210.

Palevitch, D., Yaniv, Z., Dafni, A., Friedman, J., 1986. **Medicinal plants of Israel: an ethnobotanical survey**. In: Cracker, L., Simon, J. (Eds.), Herbs, Spices and Medicinal Plants. Oryx Press, Phoenix, pp. 281–345.

Paltoo D, Woodson K, Taylor P et al. Pro l2Ala polymorphism in the peroxisome proliferator activated receptor gamma (PPAR-gamma) gene and risk of prostate cancer among men in a large cancer prevention study. **Cancer Lett** 2003; 191: 67–74.

Pan SY, Johnson KC, Ugnat AM et al. Association of obesity and cancer risk in Canada. **Am J Epidemiol** 2004; 159: 259–268

Papageorgiou V, Gardeli C, Mallouchos A, Papaioannou M, Komaitis M (2008) Variation of the chemical profile and antioxidant behavior of Rosmarinus officinalis L. and Salvia fruticosa Miller grown in Greece. **J Agric Food Chem** 56:7254–7264

Parry, J.W., 1969. **Spices: Morphology, Histology, Chemistry**. Chem Publishing Company, New York.

Parnham, M.J., Kesselring, K., 1985. Rosmarinic acid. **Drugs Future** 10, 756–757.

Paton, A., 1991. **Salvia lanceolata, Labiateae: In the Kew Magazine Incorporating Curtis's Botanical Magazine**. The Royal Botanic Gardens, Kew.

Pattnaik, S., Subramanyan, V.R., Bapaji, M., Kole, C.R., 1997. Antibacterial and antifungal activity of aromatic constituents of essential oils. **Microbios** 89 (358), 39–46.

Peeters A, Barendregt JJ,Willekens F et al. Obesity in adulthood and its consequences for life expectancy: a life-table analysis. **Ann Intern Med** 2003; 138: 24–32.

Perfumi, M., Arnold, N., Tacconi, R., 1991. Hypoglycemic activity of Salvia fruticosa Mill. from Cyprus. **Journal of Ethnopharmacology** 34 (2–3), 135–140.

Pinto-Sietsma SJ, Janssen WM, Hillege HL et al. Urinary albumin excretion is associated with renal functional abnormalities in a nondiabetic population. **J Am Soc Nephrol** 2000; 11: 1882–1888.

Pitarevic, I., Kustrac, D., Kuftine, J., Blazevic, N., 1985. **Influence of ecological factors on the content and composition of the essential oil in Salvia officinalis**. In: Svendsen, B., Scheffer, J.J.C. (Eds.), Essential Oils and Aromatic Plants. Junk Publishers, Dordrecht, The Netherlands, pp.203–207.

Pitarokili D, Tzakou O, Loukis A, Harvala C (2003) Volatile metabolites from Salvia fruticosa as antifungal agents in soil borne pathogens. **J Agric Food Chem** 51:3294–3301

Pizzale L, Bortolomeazzi R, Vichi S, U ¨ beregger E, Conte LS (2002).Antioxidant activity of sage (*Salvia officinalis and S. fruticosa*) and oregano (*Origanum onites and O. indercedens*) extracts related to their phenolic compound content. **J Sci Food Agric** 82:1645–1651.

Pou KM, Massaro JM, Hoffmann U, Vasan RS, Maurovich-Horvat P, Larson MG, et al. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. **Circulation** 2007; 116:1234-41.

Prat-Larquemin L, Oppert JM, Cle ´ment K et al. Adipose angiotensinogen secretion, blood pressure and AGT M235T polymorphism in obese patients. **Obes Res** 2004; 12: 556–561.

Ramadan A, Afifi NA, Fathy MM, El-Kashoury EA, El-Naeneey EV. **Vet Med J Giza** 1994; 42:263.

Resta O, Foschino-Barbaro MP, Legari G et al. Sep-related breathing disorders, loud snoring and excessive daytime sleepiness in obese subjects. **Int J Obes Relat Metab Disord** 2001; 25: 669–675.

Reuters (WS), Premium, September 16, 1997.

Reynolds, E.F., 1996. **Martindale: The Extra Pharmacopoeia**, 31st ed. Royal Pharmaceutical Society, London.

Rhyu, H.Y., 1979. Gas chromatographic characterization of sages of various geographic origins. **Journal of Food Science** 44, 758–762.

Ribstein J, du Cailar G & Mimran A. Combined renal effects of overweight and hypertension. **Hypertension** 1995; 26: 610–615.

Rintanen H, Hakkarainen K. **Avohoidon tilastouudistuksen pilottiprojektin aineiston tietopoiminta**. Stakes, 2005. Ref Type: Report.

Rivera, D., Obon, C., Cano, F., 1994. The botany, history and traditional uses of three-lobed sage (Salvia fruticosa Miller). **Economic Botany** 48 (2), 190–195.

Rosengarten, F., 1973. **The Book of Spices**. Pyramid Books, New York.

Ross R. Atherosclerosis: an inflammatory disease. **N Engl J Med** 1999; 340: 115–126.

Ruhl CE & Everhart JE. Relationship of serum leptin concentration and other measures of adiposity with gallbladder disease. **Hepatology** 2001; 34: 877–883.

R. Castro-Martinez, D. E. Pratt, and E. E. Miller, Proc. World Conf. Emerging Technology, Fats, Oils Ind. Meeting Date 1985, Editor (s): A. Baldwin, Richard. Publisher, **Am. Oil Chem. Soc**., Champaign, Ill, 1986, 392.

Santos, F.A., Rao, V.S., 1997. Mast cell involvement in the rat paw oedema response to 1,8-cineole, the main constituent of eucalyptus and rosemary oils. **European Journal of Pharmacology** 331 (2-3), 253–258.

Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. **Proc Natl Acad Sci USA** 2003; 100:7265-70.

Saxena Y, Saxena V, Dvivedi J, Sharma RK. Evaluation of dynamic function tests in normal obese individuals. **Indian J Physiol Pharmacol** 2008; 52:375-82.

Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. **Circulation** 2000; 101:1899-906.

Schilcher, H., 1985. Effects and side-effects of essential oils. In: Svendsen, B., Scheffer, J.J.C. (Eds.), **Essential Oils and Aromatic Plants**. Junk, Dordrecht, The Netherlands, pp. 217–231.

Seres L, Lopez-Ayerbe J, Coll R et al. Cardiopulmonary function and exercise capacity in patients with morbid obesity. **Rev Esp Cardiol** 2003; 56: 594–600.

Shan B, Cai YZ, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. **Int J Food Microbiol** 2007;117:112-9.

Shelef, L.A., Naglik, O.A., Bogen, B.W., 1990. Sensitivity of some common food borne bacteria to spices, sage rosemary and all spice. **Journal of Food Science** 45, 1042–1044.

Shepherd PR & Kahn BB. Glucose transporters and insulin action. Implications for insulin resistance and diabetes mellitus. **N Engl J Med** 1999; 341: 248-257.

Shimokawa H. Primary endothelial dysfunction: atherosclerosis. **J Mol Cell Cardiol** 1999; 31:23-37.

Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. **Nat Med** 1996; 2:800-3.

Shinton R, Shipley M, Rose G. Overweight and stroke in the Whitehall study. **J Epidemiol Community Health** 1991; 45(2):138-42.

Sivropoulou A, Nikolaou C, Papanikolaou E, Kokkini S, Lanaras T, Arsenakis M (1997). Antimicrobial, cytotoxic, and antiviral activities of *Salvia fruticosa* essential oil. **J Agric Food Chem** 45:3197–3201.

Skoula M, Abbes JE, Johnson CB (2000). Genetic variation of volatiles and rosmarinic acid in populations of Salvia fruticosa Mill growing in Crete. **Biochem Syst Ecol** 28:551–561.

Slee, D. H.; A. S.; Nguyen, T. N.; Kish, M.; Lundeen, K.; Newman, M. J.; McConnell, S. J. Pyrrolpyrazinedione-Based Inhibitors of Human Hormone-Sensitive Lipase. **J. Med. Chem.**2003, 46, 1120 -1122.

Soderberg S, Ahren B, Stegmayr B et al. Leptin is a risk marker for first-ever hemorrhagic stroke in a population-based cohort. **Stroke** 1999; 30: 328–337.

Sokovic M, Tzakou O, Pitarokili D, Couladis M (2002) **Antifungal activities of selected aromatic plants growing wild in Greece**. Die Nahrung 46:317–320.

Solerte SB, Fioravanti M, Pezza N et al. Hyperviscosity and microproteinuria in central obesity: relevance to cardiovascular risk. **Int J Obes Relat Metab Disord**; 1997; 21: 417–427.

Song YM, Sung J, Davey Smith G. & Ebrahim S.. Body mass index and ischemic and hemorrhagic stroke: a prospective study in Korean men. **Stroke** 2004; 35: 831–836.

Sowers JR. Obesity as a cardiovascular risk factor. **Am J Med** 2003; 115(supplement 8A): 37S–41S.

Stahl, E., 1973. **Drug Analysis by Chromatography and Microscopy: a Practical Supplement to Pharmacopoeias**. Ann Arbor Science, Ann Arbor, MI.

Stanley AH, Demissie K, Rhoads GG. Asthma development with obesity exposure: observations from the cohort of the National Health and Nutrition Evaluation Survey Epidemiologic Follow-up Study (NHEFS). **J Asthma** 2005; 42:97-9.

Stamler R, Stamler J, Riedlinger WF, Algera G, Roberts RH. Weight and blood pressure. Findings in hypertension screening of 1 million Americans. **JAMA** 1978; 240(15):1607-10.

Steinberg HO, Chacker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction: implications for the syndrome of insulin resistance. **J Clin Invest** 1996; 97:2601-10.

Stevens J, Cai J, Pamuk ER et al. The effect of age on the association between body-mass index and mortality. **N Engl J Med** 1998; 338: 1-7.

Sturm R. Increase in clinically severe obesity in the United States, 1986-2000. **Arch Intern Med** 2003; 163: 2146-2148.

Stuveling EM, Hillege HL, Bakker SJ et al. C-reactive protein is associated with renal function abnormalities in a non-diabetic population. **Kidney Int** 2003; 63: 654–661.

Suk SH, Sacco RL, Boden-Albala B et al. Northern Manhattan Stroke Study. Abdominal obesity and risk of ischemic stroke: the Northern Manhattan stroke study. **Stroke** 2003; 34: 1586–1592.

Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. **Circulation** 2000;101: 948-54.

Thomas Heitzer, MD; Titus Schlinzig, BS; Karoline Krohn, BS; Thomas Meinertz, MD; Thomas Münzel, MD. **Circulation**. 2001; 104: 2673.

Thomson A. B., Schoeller C., Keelan M., Smith L., Clandinin M. T., Can. **J. Physiol. Pharmacol.** 1993, 71:531-555.

Todorov, S., Philianos, S., Petkov, V., Harvala, C., Zamfirova, R., Olimpiou, H., 1984. Experimental pharmacological study of three species from genus *Salvia*. **Acta Physiologica et Pharmacologica Bulgarica** 10 (2), 13–20.

Tornberg SA & Carstensen JM. Relationship between Quetelet's index and cancer of breast and female genital tract in 47,000 women followed for 25 years. **Br J Cancer** 1994; 69: 358–361.

Tsunoda S, Kamide K, Minami J & Kawano Y. Decreases in serum uric acid by amelioration of insulin resistance in overweight hypertensive patients: effect of a low-energy diet and an insulin-sensitizing agent. **Am J Hypertens** 2002; 15: 697–701.

Tucker, A. O., Maciarello, M. J., Howell, J. T., 1980. Botanical Aspects of commercial sage. **Econ. Bot.** 34, 16} 19.

Ulubelen A., Isidatici, S., Ozturk, S., 1988. A new Flavone from Salvia triloba L. f (Labiatae). **J. Pharm. Sci.** 57, 1037–1038.

Ulubelen A., **Planta Med** 1990; 56:82.

Valencia-Flores M, Orea A, Castano VA et al. Prevalence of sleep apnea and electrocardiographic disturbances in morbidly obese patients. **Obes Res** 2000; 8: 262–269.

Valnet, J., 1990. **Aromatherapie**, Maloine. S.A. Publishers, Paris, pp. 338–343.

Van Harmelen V, Elizalde M, Ariapart P et al. The association of human adipose angiotensinogen gene expression with abdominal fat distribution in obesity. **Int J Obes Relat Metab Disord** 2000; 24: 673–678.

Verzar-Petri, G., Then, M., Meszaros, S., 1985. Formation of essential oil in clary sage under different conditions. In: Svendsen, B., Scheffer, J.J.C. (Eds.), **Essential Oils and Aromatic Plants**. Junk, Dordrecht, The Netherlands, pp. 199–202.

VOKOU, D. 1992. The allelopathic potential of aromatic shrubs in phryganic (East Mediterranean) ecosystems, pp. 304–319, in S. J. H. Rizvi and V. Rizvi (eds.). **Allelopathy: Basic and Applied Aspects**. Chapman & Hall, London.

Wasan, K M. and Looije, NA. **J. Pharm. Pharmaceut. Sci.** 2005 8:259-271.

Wassef N, Sidhom G, Zakareya el-K & Mohamed el-K. Lipoprotein(a) in android obesity and NIDDM. **Diabetes Care** 1997; 20: 1693–1696.

Waterman, P.G., 1993. **The chemistry of volatile oils**. In: Hay, R.K.M., Waterman, P.G. (Eds.), Volatile Oil Crop. Long-man Scientific & Technical, England, pp. 41–61.

Wellman NS, Friedberg B. Causes and consequences of adult obesity: health, social and economic impacts in the United States. **Asia Pac J Clin Nutr** 2002; 11(Suppl. 8):S705-9.

Weyer C, Funahashi T, Tanaka S et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hiperinsulinemia. **J Clin Endocr Metab** 2001; 86: 1930–1935.

WHO Consultation on Obesity, Obesity: preventing and managing the global epidemic. **WHO Technical Report Series** 894, Geneva, 2000.

WHO/FAO Joint Consultation. Diet, nutrition and the prevention of chronic diseases: Report of a joint WHO/FAO expert consultation. Geneva: WHO; 2003.

Williams PT & Krauss RM. Associations of age, adiposity, menopause, and alcohol intake with low-density lipoprotein subclasses. **Arterioscler Thromb Vasc Biol** 1997; 17: 1082–1090.

Wilson PW, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. **Arch Intern Med** 2002;162:1867—72.

Wing RR, Mathews KA, Kuller LH, Meilahn EN, Plantinga P. Waist to hip ratio in middle-aged women. Associations with behavioral and psychological factors and with changes in cardiovascular risk factors. **Arterioscler Thromb** 1991; 11:1250-7.

Wu Y, Zhou B, Tao S et al. Prevalence of overweight and obesity in Chinese middle-aged populations: current status and trend of development. **Chin J Epidemiol** 2002; 23: 11–15.

Yamauchi T, Kamon J, Waki H et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. **Nat Med** 2001; 7: 941-946.

Young T, Palta M, Dempsey J et al. The occurrence of sleep-disordered breathing among middle-aged adults. **N Engl J Med** 1993; 328: 1230–1235.

Young T, Shahar E, Nieto FJ et al. Predictors of sleep-disordered breathing in community-dwelling adults: the sleep heart health study. **Arch Intern Med** 2002; 162: 893–900.

Yudkin JS, Kumari M, Humphries SE & Mohamed-Ali V. **Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis** 2000; 148: 209-214.

Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, et al. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. **Lancet** 2005; 366(9497):1640-9.

Appendix

A.1 Pancreatic Lipase (PL) solution preparation:

Add tris-buffer solution (PH=7.4) to 25 mg lipase up to 50 ml.

Stirring for half hour at rpm 250.

Centrifugation for five minutes at rpm 4000

Take the supernatant and put it in ice.

(Note : freshly prepared)

A.2 Plant extract solution preparation:

Weigh about 10 mg of plant extract in an eppendorf tube.

Dissolve in DMSO to obtain a final concentration 10mg/ml solution.

Dissolving by vortex.

A.3 Substrate solution preparation:

Put 50 µl substrate (PNPB) in eppendorf tube, complete up to 1 ml with acetonitrile

A.4 Tris-buffer solution (PH: 7.4) preparation:

Transfer 0.303g of tris-base (2.5 mM) and 5.85g NaCL (0.1 M) to 500 ml volumetric flask.

Add distilled water and stirring.

Add 1 M HCL solution according to target PH (PH=7.4).

تقيم خلاصة نبتة الميرميه لاختبار فعاليتها كعامل مخفض للدهنيات الثلاثية

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ملخص

الخلفية : الميرميه عباره عن نبته تنتمي الى عائلةالشفافيات ولديها الكثير من الخصائص المهمه،مثل: الخصائص المضادة للأكسدة ، مضادة للبكتيريا ، مضاد للالتهابات ، مضاد للفطريات ، مضاد للفيروسات ، عامل مخفض لسكر الدم ، وعامل مخفض للدهنيات الثلاثية . وقد صممت هذه الدراسة لاختبار فعاليتها كعامل مخفض للدهنيات الثلاثية في الفئران بعد عمل استخلاص للنبته باستخدام الميثانول.

الطرق المتبعه : تم تقييمها عن طريق اعطائها الى الفئران عن طريق الفم (التركيز المستخدم 750 ملغم / كغم من وزن جسم الفأر) ومن ثم تم أخذ عينات دم من الفئران لفحص كمية الدهون الثلاثية الموجوده في دمه جنبا إلى جنب مع التحليل في المختبر.

النتائج : أظهرت الدراسه انخفاض كبير في إجمالي الدهون الثلاثية مما يدل على انه ثبط عمل أنزيم الليبيز (يعمل على تحطيم الدهون الى مكونات ابسط حتى يمتصها الجسم).كما تم حساب تركيز خلاصة الميرميه التي تعمل على تثبيط انزيم الليبيز الى النصف (IC50) ووجد انه يساوي 100.9 ميكروغرام / ميليلتر ، هذا وكان الأورليستات (دواء معروف باسم زينيكال وهو يثبط انزيم الليبيز ايضا) يستخدم كمرجع .

الاستنتاجات : ويستنتج من ذلك أن الميرميه لها تأثير مثبط لأنزيم الليبيز مثل دواء الأورليستات مما يساعد على تخفيض كمية الدهنيات الثلاثيه الممتصه من الطعام وهذا يساعد على تخفيض وزن الجسم.